JOURNAL OF AGRICULTURAL RESEARCH

VOL. 55 WASHINGTON, D. C., OCTOBER 1, 1937 NO. 7

CROSS TRANSFER OF MINERAL NUTRIENTS IN THE TOBACCO PLANT[1]

By J. E. McMURTREY, JR.

Senior physiologist, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Uniformity of growth in plants is a matter of considerable theoretical as well as practical interest. In studies concerning the mineral nutrition of tobacco, in regard to distinctive growth effects manifested by this plant (*6*, *7*, *10*, *11*),[2] it was frequently observed under field conditions (*12*) that one-half of a leaf exhibited characteristic deficiency effects when such effects were not so evident on the other half. The observed effects appeared to be due to a failure of cross transfer of nutrients even between halves of an individual leaf. These observations furnished the basis for the investigations reported in this paper. The problem appears to have some bearing on the field placement of fertilizers, particularly separate placements of the different ingredients and deferred side dressings.

The work of Gile and Carrero (*8*, *9*) has definitely shown a reduction in growth and decreased absorption when the roots are divided between different containers so that some portion or all of the root systems are supplied with an incomplete nutrient solution. These authors did not describe in detail the appearance of the plants grown in tests. The studies by Auchter (*2*) with woody plants support the idea that there is little or no cross transfer of nutrients but a free cross transfer of water in woody plants. Auchter found that there was a unilateral distribution, that is, the nitrate applied on one side of the tree produced responses that were evident on the side of the tree to which the application was made. Caldwell (*3*, *4*) concluded that salts and carbohydrates were translocated up or down the plant stem but that there was little cross transfer of materials. Localization of day-length effects reported by Garner and Allard (*5*) further emphasizes the failure of translocation. These workers found striking localization of the principle responsible for the initiation of flowering in plants. These observations are difficult to interpret from the standpoint of plant anatomy if there be any considerable movement through the vascular bundles, since Artschwager (*1*) has reported more or less complete anastomosis of these bundles, which should operate to mix all materials moving through these channels.

[1] Received for publication Jan. 18, 1937; issued October 1937.

[2] Reference is made by number (italic) to Literature Cited, p. 481.

PROCEDURE

In an effort to determine why only one side of a leaf may manifest distinctive symptoms due to a deficiency of any one of the essential elements necessary for growth, the roots of the tobacco plant (*Nicotiana tabacum* L.) were divided into approximately two equal portions, each of which was placed in a 1-liter Pyrex beaker, tall form. The plant was held in position by a varnished wooden board provided with suitable holes and a stake to which the plant was tied. Early attempts to produce the desired result were not successful, apparently because fibrous roots developing near the junction of the beakers tended to mix the two solutions through a wicklike effect. This complication is particularly serious in solution cultures but should not be operative in sand or soil cultures. In an effort to overcome this difficulty in studies conducted during 1930, the stalk was split to about 1 inch above the root crown. Under these conditions it was possible to obtain definite effects, which are described later.

Since the procedure just described is open to the objection that the stele would be divided and cross transfer, therefore, prevented, further studies were undertaken. The fibrous roots were removed to avoid the mixing of solutions that occurred in the early trials. Solutions were prepared like those used in previous studies (*11*), from each of which one of the following elements was withheld in turn: Nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, boron, iron, and manganese. After having been disinfected with silver nitrate according to the procedure previously described (*11*), plants were transferred on April 1, 1936, from soil to duplicate culture solutions from which, in each instance, one of the above elements had been withheld, except that in one group all these elements were included in both containers. Each container was aerated by bubbling with compressed air at approximately 1 liter per hour. On May 17 all solutions were renewed, a complete solution being added to the container for one-half of the roots of each plant of the incomplete groups and to the containers for both halves in the complete group. Additional nutrient was subsequently added at intervals of 2 weeks.

The procedure of growing the plants in incomplete nutrient solutions until characteristic deficiency symptoms developed, as previously described (*11*), was adopted because it appeared to give the most logical approach to the problem. It had been previously determined that the development of symptoms was slower than was the subsequent recovery. Such procedure caused the plant to develop symmetrically in regard to root and top growth, permitting later changes to produce sharper contrasts.

RESULTS AND DISCUSSION

Except in the case of manganese, all cultures were manifesting characteristic deficiency effects at the time provision was made for supplying one-half of the roots with a complete nutrient solution (*11*). Two weeks later it was evident that cross transfer was not rapid enough to give uniform recovery. This condition extended even to a half of the individual leaf, as shown in figure 1, where a large portion of the leaf on one side of the midrib has recovered its normal green color while the remaining half is chlorotic because of a deficient supply of magnesium. This appears to be the first reported instance

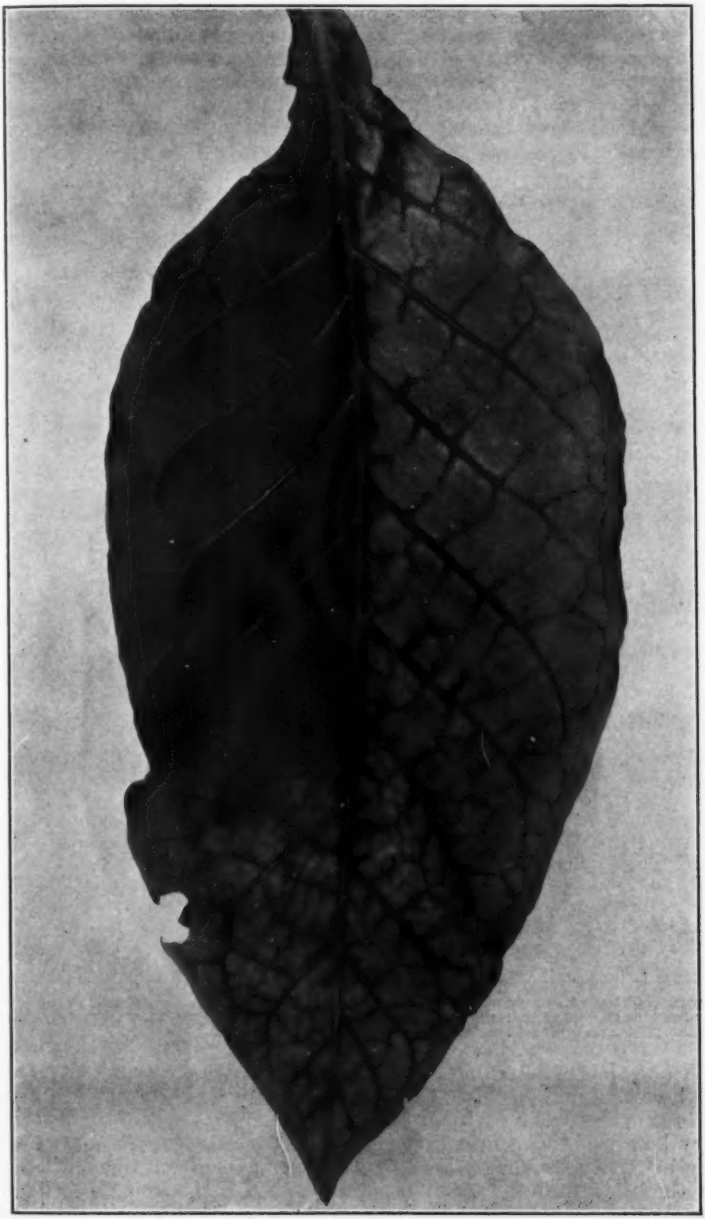

FIGURE 1.—Tobacco leaf manifesting one-sided recovery caused by supplying the element magnesium to one-half of the root system and withholding it from the other half.

where a failure of cross transfer of a mineral nutrient in the individual leaf has been shown to occur. This situation was more or less common to deficiency symptoms manifested for all elements except manganese. Possibly the experiment was not of sufficient duration to induce effects from shortages of this element. The unilateral leaf manifestation was not so commonly observed in calcium and boron deficiency combinations.

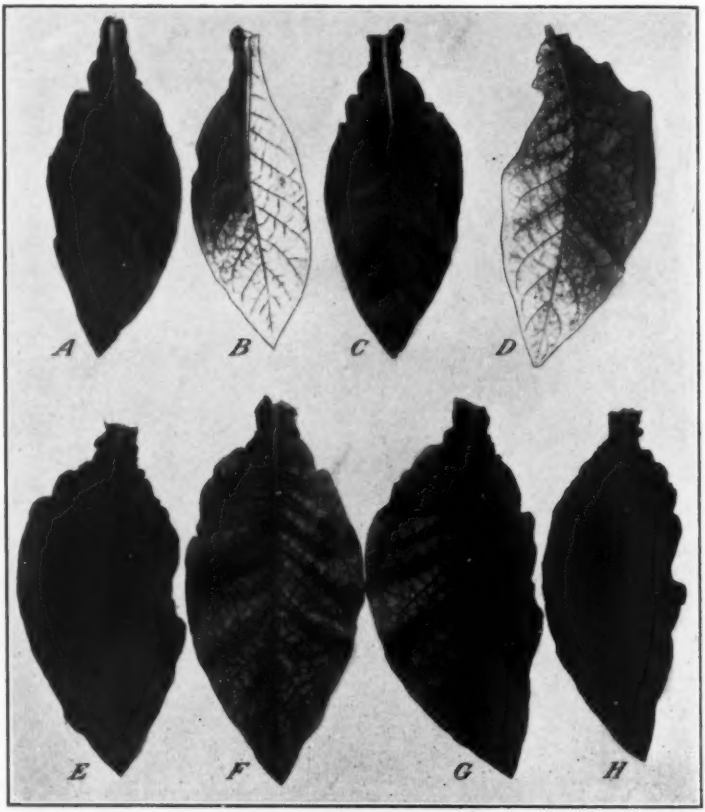

FIGURE 2.—Tobacco leaves showing distribution of recovered and chlorotic leaves (*A–H*) removed from a plant from the base, *A*, upward to *H*, when magnesium was supplied to one-half of the root system and withheld from the other half.

A question of some interest is whether recovery may be unilateral in the individual leaf, but this appears not to be the case. In some instances the entire leaf may show bilateral recovery, or again there may be a leaf that shows no recovery. Nor does there appear to be any definite relationship between the arrangement of affected leaves on the stalk and the 3/8 phyllotaxy of the tobacco plant. These observations are supported by figure 2, in which the leaves *A* to *H* are arranged as they occurred on the stalk, beginning at the base.

In this instance magnesium was supplied to only one-half of the roots. This same observation held true in greater or less degree for all combinations tested. In many instances there does appear to be unilateral or half-leaf manifestation, but the leaf halves concerned do not always occur on the same side. This is shown, for example, in figure 2, for *B* shows recovery on one-half while *D* manifests recovery on the opposite half.

Conceivably it would be possible so to subdivide the roots that there would be a symmetrical effect, but such a result was never

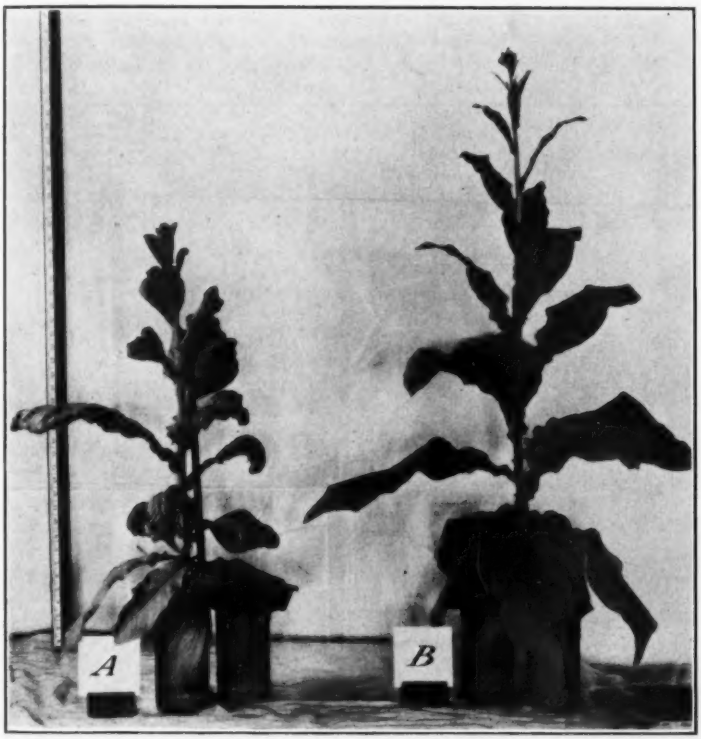

FIGURE 3.—Tobacco plants grown in solution cultures. *A*, Calcium added to container on left after being withheld for a time. This element was not added to container on right for period of observation. *B*, Complete nutrient solution supplied to both containers for the entire period of observation.

observed in the combinations tested. It appears that no systematic arrangement can be expected, since it would be highly improbable that by mere chance the roots could be divided in such a manner as to produce such a result. The plants made additional growth following their transfer to the conditions adapted to study of recovery. This growth also manifested the failure of cross transfer, indicating that there would have been this failure even if the roots had been grown half in complete and half in incomplete solutions for the entire period. The experiment was discontinued June 18, approximately

1 month after the transfer was made, and none of the plants manifested symmetrical growth when any particular element was supplied to only one-half the root system.

Failure of cross transfer of an essential nutrient results in a one-sided or twisted growth, as shown in figure 3, *A*, where calcium was not supplied during the early growth but later was supplied to the half of the root system nearest the letter *A*. It can be observed in this plant that the increase in size of the leaves is not unilateral in its distribution. Complete recovery from symptoms induced by calcium deficiency is not possible, since there is an actual break-down of the meristematic tissues; but it is apparent that certain leaves have increased in size, even though the leaf tissues are missing at the tips and margins. The control plant (*B*), in which calcium has

FIGURE 4.—Tobacco plants grown in solution cultures. *A*, Sulphur added to container on left after being withheld for a time. Container on right received no added sulphur. *B*, Complete nutrient solution supplied to both containers.

been supplied to the entire root system during the period of observation, manifests symmetrical development of both root and top growth.

Unilateral distribution of mineral nutrients does appear to occur in some instances, as indicated by symptom manifestations. It was characteristic of those cultures in which sulphur was not supplied for a time and then was added to the container in which one-half of the roots were growing (fig. 4, *A*) that rapid recovery was exhibited on the side of the plant to which the element was supplied. Again, it is evident that the lower leaf on plant *A* manifests a one-sided growth. In figure 4, *A*, the container to the right has received no added sulphur, and it is evident that this half of the plant is stunted. There was also a decided difference in the shade of green, which failed to register in the photograph. The leaves and even the leaf halves that manifested sulphur deficiency were a decidedly lighter

green. The control plant (*B*) is normal in appearance and symmetrical in development.

It has been observed under field conditions (*12*) that leaf halves manifest deficiency symptoms, and it would appear that failure of cross transfer is the explanation. It is conceivable that one portion of the root zone in which the plant is feeding is relatively more deficient in an element than another portion and that, owing to failure of cross transfer in the plant, one-half of the leaf would manifest more pronounced symptoms than the other half. In view of the fact that this situation has been observed to occur under field conditions, studies in fertilizer placement should take into account this phase of the problem. Placement to one side of the row and the application of different ingredients in separate, widely separated bands or positions in relation to the root system of the plant may not be desirable practices. The method of delayed side dressings should include ample provision for adequate distribution in relation to the root zone of the plant in question. The results presented in this paper appear to offer a logical basis for explaining the more efficient action of the fertilizer when placed near the plant in the region of maximum root development.

SUMMARY

It has been possible to reproduce experimentally, on one-half of an individual leaf, symptoms of nitrogen, phosphorus, potassium, magnesium, sulphur, and iron deficiency by withholding the element from a portion of the root system. Failure of cross transfer of the essential nutrients did not always manifest distinctive effects unilaterally on the individual leaf, and the plant in some instances showed an entire leaf normal while an adjacent leaf bilaterally manifested symptoms of deficiency. A twisted or one-sided growth also resulted from deficiency of each of the above-mentioned elements and also of calcium or boron when they were not supplied to the entire root system.

There was not always a unilateral distribution of the symptoms in relation to the root and top growth when the roots were divided so as to supply a given element to one-half of the root system and withhold it from the other half. Depending apparently upon root and top-growth relations, it appears to be merely a matter of chance as to what type of growth manifestation is obtained.

The possible relation of these findings to fertilizer placement under field conditions is pointed out.

LITERATURE CITED

(1) ARTSCHWAGER, E. F.
1918. ANATOMY OF THE POTATO PLANT, WITH SPECIAL REFERENCE TO THE ONTOGENY OF THE VASCULAR SYSTEM. Jour. Agr. Research 14: 221–252, illus.

(2) AUCHTER, E. C.
1923. IS THERE NORMALLY A CROSS-TRANSFER OF FOODS, WATER, AND MINERAL NUTRIENTS IN WOODY PLANTS? Md. Agr. Expt. Sta. Bull. 257, pp. 33–62.

(3) CALDWELL, J.
1930. STUDIES IN TRANSLOCATION; I. MOVEMENT OF FOOD MATERIALS IN THE SWEDE TURNIP. Roy. Soc. Edinb. Proc. 50: 130–141, illus.

(4) CALDWELL, J.
1930. STUDIES IN TRANSLOCATION; II. THE MOVEMENT OF FOOD MATERIALS IN PLANTS. New Phytol. 29: 27–43, illus.
(5) GARNER, W. W., and ALLARD, H. A.
1925. LOCALIZATION OF THE RESPONSE IN PLANTS TO RELATIVE LENGTH OF DAY AND NIGHT. Jour. Agr. Research 31: 555–566, illus.
(6) ——— McMURTREY, J. E., JR., BACON, C. W., and MOSS, E. G.
1923. SAND DROWN, A CHLOROSIS OF TOBACCO DUE TO MAGNESIUM DEFICIENCY, AND THE RELATION OF SULPHATES AND CHLORIDS OF POTASSIUM TO THE DISEASE. Jour. Agr. Research 23: 27–40, illus.
(7) ——— McMURTREY, J. E., JR., BOWLING, J. D., JR., and MOSS, E. G.
1930. MAGNESIUM AND CALCIUM REQUIREMENTS OF THE TOBACCO CROP. Jour. Agr. Research 40: 145–168, illus.
(8) GILE, P. L., and CARRERO, J. O.
1917. ABSORPTION OF NUTRIENTS AS AFFECTED BY THE NUMBER OF ROOTS SUPPLIED WITH THE NUTRIENT. Jour. Agr. Research 9: 73–95, illus.
(9) ——— and CARRERO, J. O.
1921. ASSIMILATION OF NITROGEN, PHOSPHORUS, AND POTASSIUM BY CORN WHEN NUTRIENT SALTS ARE CONFINED TO DIFFERENT ROOTS. Jour. Agr. Research 21: 545–573, illus.
(10) McMURTREY, J. E., JR.
1929. THE EFFECT OF BORON DEFICIENCY ON GROWTH OF TOBACCO PLANTS IN AERATED AND UNAERATED SOLUTIONS. Jour. Agr. Research 38: 371–380, illus.
(11) ———
1933. DISTINCTIVE EFFECTS OF THE DEFICIENCY OF CERTAIN ESSENTIAL ELEMENTS ON GROWTH OF TOBACCO PLANTS IN SOLUTION CULTURES. U. S. Dept. Agr. Tech. Bull. 340, 43 pp., illus.
(12) MOSS, E. G., McMURTREY, J. E., JR., LUNN, W. M., and CARR, J. M.
1927. FERTILIZER TESTS WITH FLUE-CURED TOBACCO. U. S. Dept. Agr. Tech. Bull. 12, 59 pp., illus.

PHYSIOLOGIC RACES OF TILLETIA TRITICI AND T. LEVIS[1]

By H. A. RODENHISER, *pathologist*, and C. S. HOLTON, *associate pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Since Faris (*3*)[2] obtained strong indications of physiologic specialization in *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn, pathogenic differences between races of both species have been studied by a number of investigators in the United States and Canada (*1, 2, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15*). In these experiments the methods used have been more or less adapted to local conditions, and this has resulted in a lack of uniformity in the use of differential hosts and in the system of numbering distinct races. Consequently it is to be expected that a number of the physiologic races that have been identified by different investigators will be duplicates. The reports of these experiments do, however, serve as definite evidence that physiologic races of these fungi have become established in the important wheat-growing areas of the United States. The significance of this fact with regard to the problem of bunt control needs no extensive elaboration.

Certain wheat varieties have been distributed in the past that had proved to be resistant to bunt prevalent in the vicinities where the varieties were developed. However, when these varieties were grown commercially they were found to be susceptible. In most instances outbreaks of bunt in such varieties have been definitely associated with the appearance of previously undescribed races of the two species. Obviously, then, if a breeding program for the development of smut-resistant varieties of wheat is to be successful it is essential to know the number, distribution, and prevalence of physiologic races. To obtain this information more rapid progress will be made if a standard system for race identification is developed and generally adopted. The present paper is intended as a first step in this direction.

MATERIALS AND METHODS

Thirty-one collections of *Tilletia tritici* and 34 of *T. levis* have thus far been given race numbers by various investigators in this country. Of this group 24 of the numbered races of the former and 29 of the latter were obtained for testing by the writers. In addition, a number of miscellaneous collections of both species were tested.

Ten varieties each of winter and of spring wheat were used as differential hosts. The winter wheats were Hybrid 128 (C. I.[3] 4512), Ridit (C. I. 6703), Oro (C. I. 8220), Albit (C. I. 8275), Hohenheimer (C. I. 11458), Hussar (C. I. 4843), Minhardi (C. I. 5149), White Odessa (C. I. 4655), Martin (C. I. 4463), and Turkey (C. I. 6175). In the final analysis of data only the first six of these were retained as differential hosts. The other four were not considered for the following reasons: Minhardi was found to be similar to Hybrid 128 in its reaction to all races and collections tested; White Odessa was similar to

[1] Received for publication Jan. 18, 1937; issued October 1937. Investigations conducted in cooperation with the West Virginia, Minnesota, Montana, Washington, and Oregon Agricultural Experiment Stations.

[2] Reference is made by number (italic) to Literature Cited, p. 495.

[3] C. I. denotes accession number of the Division of Cereal Crops and Diseases (formerly Office of Cereal Investigations).

Albit in this respect; no pathogenically distinct races could be differentiated on Martin that were not equally well differentiated on either Albit or Hussar; Turkey (C. I. 6175) was not consistent in its reaction to certain races when grown under a fairly wide range of environmental conditions. The spring wheats were Ulka (C. I. 11478), Marquis (C. I. 3641), Canus (C. I. 11637), Mindum (C. I. 5296), Hope × Ceres (C. I. 11432), Ruby (C. I. 6047), Garnet (C. I. 8188), Thatcher (C. I. 10003), Golden Ball (C. I. 11477), and Vernal emmer (C. I. 3686). In the analysis of data only the first four of these were retained as differential hosts. Certain races could be identified by their reaction on the latter six varieties, but none that were not equally well differentiated on the Ulka, Marquis, Canus, and Mindum combination.

Seed of the differential hosts was treated with formaldehyde according to the standard dip method, thoroughly washed with water to remove all traces of formaldehyde, and allowed to dry. Powdered inoculum from each collection was then applied to each lot of seed at the rate of approximately 0.5 g to 100 g of seed. The inoculated seed was planted at the rate of 5 g to the row in duplicate systematically distributed 6-foot rows. The inoculum of each race to be used in the succeeding year's test was collected on the same date and subsequently stored at room temperature in the laboratory. In preparing the inoculum and in inoculating and planting the seed, adequate precautions were observed to prevent mixing of the spores of different species and races. The smut percentages obtained were based on counts of 300 heads per row at Bozeman, Mont., and counts on the total number of heads per row at Kearneysville, W. Va., and Pullman, Wash. There were approximately 200 heads per row at Kearneysville and 400 per row at Pullman.

In the analysis of these data only those collections of smut possessing fairly wide differences in pathogenicity were considered as distinct races. With the present technique for separating races of *Tilletia*, environmental conditions are not accurately controlled nor are they duplicated at different stations and in different years. It is therefore impossible to make such fine distinctions as have been made in the rust fungi, in the study of which the environmental conditions affecting both the host plant and the fungus may be easily controlled. When a sufficiently large number of tests have been made under controlled conditions or under different environmental conditions in sufficient number to permit a statistical analysis of the results, smaller differences in pathogenicity may possibly be found to be significant. Until then, classifications of races of *Tilletia* are necessarily more or less arbitrary, and it would seem logical that separations should be made only where the differences in pathogenicity are fairly wide and reasonably consistent. In previous work relatively narrow limits in infection range classes have been considered significant for the purpose of separating physiologic races. As a result, four to six classes have been used by various investigators. However, in the present tests variations between results with the same smut collections at different stations in 1 year and in the results from replications at the same station were frequently great enough to interfere with the use of a classification with more than three infection classes as follows:

0–10 percent infection = resistant class (R).
11–40 percent infection = intermediate class (I).
41–100 percent infection = susceptible class (S).

In establishing these classes consideration has been given to the tendency toward variation in smut percentages produced by the same races on the same varieties, in different replications, in different years, and in different localities. It is clear from the data in table 1 that within the resistant class the variability in percentages of infection is small and that the upper limit for the class should be approximately 10 percent. These data also indicate that as the degree of resistance decreases the variability in the percentages of infection obtained with any one collection of smut increases. For example, as recorded in table 1, the percentage of smut on Albit inoculated with T–4 varied from 10 to 44 percent depending on the locality or the year the test was made. It is to allow for such variations in percentages as these that the limits for the intermediate and susceptible classes have been established.

Tests for the differentiation of physiologic races are considered satisfactory in these experiments only when 60 percent or more smut developed in the generally susceptible varieties, Hybrid 128 and Ulka. Accordingly no data are presented from tests in which infection in these varieties failed to reach this figure. The classification as recorded is based on results obtained in tests with winter wheat in 1 of the 4 years the experiments were made at Kearneysville, W. Va.; in 1 of the 3 years at Bozeman, Mont.; and both of the 2 years at Pullman, Wash. In the tests with spring wheat, satisfactory data for race differentiation were obtained in 1 of the 3 years tests were made at Bozeman, and 1 of the 2 years at Pullman. No significant data were obtained in two seasons the tests were made with spring wheat at St. Paul, Minn., because of drought conditions, and with winter wheat at Corvallis, Oreg., because of poor stands.

Reactions that differentiate physiologic races are indicated in table 3 by the bold-faced type, and the letter T or L is prefixed to the race numbers to denote the smut species *Tilletia tritici* and *T. levis*, respectively.

RESULTS

IDENTIFICATION OF PHYSIOLOGIC RACES

At the present time tests adequate to warrant classification have been made with 24 collections of *Tilletia tritici* and 28 of *T. levis*. From these collections 11 pathogenically distinct races of *T. tritici* and 8 of *T. levis* have been identified and assigned letters and numbers T–1 to T–11 and L–1 to L–8, respectively. The average percentages of smut produced by these races at different stations and in different years are recorded in table 1. The sources of inoculum for these tests, together with the assigned race numbers, are recorded in table 2. The reactions that serve to differentiate the races of *T. tritici* and *T. levis* are presented in table 3.

In previous investigations the identification of physiologic races of *Tilletia tritici* and *T. levis* has been based upon their reaction on either winter or spring wheats alone. The results of the present studies emphasize the value of using both winter and spring varieties as differential hosts. For example, races L–1, L–2, and L–3 (table 3) are differentiated on spring wheats alone, races L–6 and L–8 on winter wheats alone, and races L–4 and L–5 on a combination of both winter and spring wheat varieties. Race L–7 is clearly differentiated by its reaction on either the spring or winter wheat varieties. Similar results were obtained with races of *T. tritici* (table 3).

TABLE 1.—*Percentages of bunt obtained in 6 varieties of winter wheat and 4 varieties of spring wheat inoculated with 11 physiologic races of Tilletia tritici and 8 physiologic races of T. levis at Bozeman, Mont., Pullman, Wash., and Kearneysville, W. Va., 1934–36*

TILLETIA TRITICI

Physiologic race no.	Year tested	Percentage of bunt in varieties at localities indicated																			
		Hybrid 128 (C. I. 4512)				Ridit (C. I. 6703)				Oro (C. I. 8220)				Hohenheimer (C. I. 11458)				Hussar (C. I. 4843)			
		Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Kearneysville	Average
T-1	1934	69			80	2			3	4			2	1			1.0	5			2.0
	1935		82	84			2.0	5			2.0	1			1.0	0			0.4	1.0	
	1936		85				3.0				1.0				.0				1.0		
T-2	1934	64			84	0			2	0			1	0			.1	2			1.0
	1935		81	95			.0	5			2	1			.3	0			.3	.3	
	1936		93				1.0				2.0				.0				.0		
T-3	1934	75			86	2			7	4			4	1			.3	3			1.0
	1935		92	86			8.0	4			9.0	2			.0	0			1.0	.0	
	1936		92				12.0				1.0				.0				.2		
T-4	1934	90			91	1			3	4			3	1			.3	3			1.0
	1935		93	90			3.0	5			5.0	2			.0	0			1.0	.4	
	1936		92				2.0				1.0				.0				.0		
T-5	1934	87			91	9			8	8			5	0			1.0	9			4.0
	1935		91	93			10.0	6			7.0	3			.0	2			2.0	3.0	
	1936		93				5.0				3.0				.0				1.0		
T-6	1934	71			82	2			2	3			2	1			.3	5			3.0
	1935		91	94			1.0	3			3.0	1			.0	0			5.0	.4	
	1936		71				2.0				1.0				.0				.4		
T-7	1934	79			83	3			3	2			2	2			1.0	26			33.0
	1935		90	81			5.0	1			3.0	2			.0	0			30.0	42.0	
	1936																				
T-8	1934	78			89	8			6	2			4	3			1.0	34			53.0
	1935		95	86			5.0	6			5.0	3			.0	1			57.0	78.0	
	1936		95				5.0				5.0				.0				42.0		
T-9	1934		90		87		2.0		1		3.0		2		34.0		32.0		.0		1.0
	1935																				
	1936		84				.1				1.0				29.0				1.0		
T-10	1934		91		90		1.0		1		5.0		3		69.0		67.0		0		.0
	1935		86				1.0				2.0				60.0				.0		
	1936		93				2.0				2.0				73.0				.0		
T-11	1934				84				56				2				.0				3.0
	1935		90				55.0				1.0				.0				1.0		
	1936		78				56.0				3.0				.0				4.0		

Physiologic race no.	Year tested	Percentage of bunt in varieties at localities indicated															
		Albit (C. I. 8275)				Ulka (C. I. 11478)			Marquis (C. I. 3641)			Canus (C. I. 11637)			Mindum (C. I. 5296)		
		Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Average	Bozeman	Pullman	Average	Bozeman	Pullman	Average	Bozeman	Pullman	Average
T-1	1934	0															
	1935		0.4	0	0.1	73	95	84	33	38	36	0.4	1.0	1.0	18	1	10
	1936		.0														
T-2	1934	1															
	1935		.0	2	1.0	83	94	89	1	2	2	.2	1.0	1.0	74	66	70
	1936		.1														
T-3	1934	0															
	1935		1.0	1	1.0	72	98	85	58	46	52	50.0	46.0	48.0	18	14	16
	1936		.0														
T-4	1934	13															
	1935		18.0	44	21.0	92	91	92	57	39	48	6.0	.0	3.0	22	3	13
	1936		10.0														
T-5	1934	16															
	1935		22.0	13	14.0	86	96	91	71	48	60	51.0	54.0	53.0	22	12	17
	1936		3.0														
T-6	1934	70															
	1935		90.0	92	78.0	92	85	89	41	42	42	3.0	.0	2.0	25	3	14
	1936		58.0														
T-7	1934	69															
	1935		89.0	87	82.0	92	97	95	52	56	54	21.0	15.0	18.0	26	9	18
	1936																
T-8	1934	79															
	1935		88.0	92	88.0	70	91	81	46	46	46	52.0	43.0	48.0	25	7	16
	1936		91.0														
T-9	1934		.0														
	1935				4.0	93	88	91	28	29	29	1.0	.2	1.0	27	23	25
	1936		7.0														
T-10 [1]	1934		4.0														
	1935		.3		2.0												
	1936		.1			11	8	10	44	21	33	.0	.1	.1	11	5	8
T-11	1924																
	1935		2.0		7.0	19	32	26	46	36	41	57.0	46.0	52.0	39	10	25
	1936		11.0														

[1] The reaction of this race on the spring wheat host testers was obtained in 1936 only.

TABLE 1.—*Percentages of bunt obtained in 6 varieties of winter wheat and 4 varieties of spring wheat inoculated with 11 physiologic races of Tilletia tritici and 8 physiologic races of T. levis at Bozeman, Mont., Pullman, Wash., and Kearneysville, W. Va., 1934–36*—Contd.

TILLETIA LEVIS

Physiologic race no.	Year tested	Percentage of bunt in varieties at localities indicated																			
		Hybrid 128 (C. I. 4512)				Ridit (C. I. 6703)				Oro (C. I. 8220)				Hohenheimer (C. I. 11458)				Hussar (C. I. 4843)			
		Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Kearneysville	Average
L–1	1934	82				2				2				3				4			
	1935		92	89	87		3.0	1	2		3.0	1	2		0.0	0	1.0		1.0	0.0	1.0
	1936		83				1.0				.3				.0				0		
L–2	1934	85				2				1				1				1			
	1935		92	89	88		4.0	3	3		2.0	1	2		.0	2	1.0		.0	.0	.3
	1936		86				2.0				2.0				.4				1		
L–3	1934	75				4				2				0				3			
	1935		89	92	87		4.0	3	4		7.0	2	5		.0	0	.0		.0	.3	1.0
	1936		92				5.0				9.0				.0	0			1.0		
L–4	1934	87				1				5				0				3			
	1935		96	83	87		5.0	3	3		5.0	7	5		.0	0	.0		13.0	2.0	5.0
	1936		81				3.0				1.0				.0				1.0		
L–5	1934																				
	1935		88	83	86		5.0	4	5		7.0	5	5		2.0	0	1.0		4.0	2.0	2.0
	1936		87				6.0				3.0				0				1.0		
L–6	1934	78				5				5				1				11			
	1935		93	89	87		5.0	4	4		7.0	3	6		.0	0	.3		19.0	22.0	15.0
	1936		88				2.0				7.0				.0				6.0		
L–7	1934	63				3				6				0				30			
	1935		85	95	84		4.0	3	5		7.0	9	8		.0	0	.0		26.0	81.0	50.0
	1936		91				8.0				10.0				.0				54.0		
L–8	1934	74				3				56				0				3			
	1935		95	92	89		7.0	10	6		78.0	67	73		.0	0	.0		1.0	2.0	2.0
	1936		95				4.0				90.0				.0				1.0		

Physiologic race no.	Year tested	Percentage of bunt in varieties at localities indicated															
		Albit (C. I. 8275)				Ulka (C. I. 11478)			Marquis (C. I. 3641)			Canus (C. I. 11637)			Mindum (C. I. 5296)		
		Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Average	Bozeman	Pullman	Average	Bozeman	Pullman	Average	Bozeman	Pullman	Average
L–1	1934	0															
	1935		0.3	0.4	0.2	73	96	85	34	39	37	1	0.0	1.0	23	20	22
	1936		.0														
L–2	1934	10															
	1935		10.0	4.0	7.0	89	95	92	60	47	54	4	.1	2.0	27	13	20
	1936		5.0														
L–3	1934	0															
	1935		1.0	.0	.3	90	96	93	42	39	41	62	61.0	62.0	26	6	16
	1936		.0														
L–4	1934	83															
	1935		90.0	86.0	87.0	81	96	89	42	36	39	3	.3	2.0	23	6	15
	1936		88.0														
L–5	1934																
	1935		68.0	73.0	72.0	89	97	93	44	47	46	62	52.0	57.0	22	30	26
	1936		76.0														
L–6	1934	28															
	1935		73.0	79.0	51.0	82	96	89	54	33	44	60	56.0	58.0	21	8	15
	1936		24.0														
L–7	1934	65															
	1935		72.0	87.0	79.0	82	89	86	40	26	33	53	48.0	51.0	40	10	25
	1936		91.0														
L–8	1934	1															
	1935		1.0	.0	1.0	72	95	84	36	43	40	60	69.0	65.0	16	12	14
	1936		.1														

TABLE 2.—*Previously numbered physiologic races and miscellaneous collections of Tilletia tritici and T. levis grouped to correspond with new race numbers*

Smut species	Physiologic race no.[1]	Source of inoculum
Tilletia tritici	T-1	Gaines' 1; Flor's 6; Reed's 3; collection from Davis, Calif.
	T-2	Holton's Vernal emmer race; Holton's Mindum race; collection from Langdon, N. Dak.
	T-3	Reed's 5; Young's Montana collection 38.
	T-4	Reed's 2.
	T-5	Reed's 4.
	T-6	Gaines' 3; Bressman's 7 and 10; Young's Montana collection 20; Rodenhiser and Stakman's Manitoba collection.
	T-7	Collection from Pullman, Wash.
	T-8	Gaines' 2; Bressman's 9; Young's Montana collection 100.
	T-9	Bressman's 8; Flor's 7.
	T-10	Collection from Lind, Wash.
	T-11	Collection from Pullman, Wash.
Tilletia levis	L-1	Reed's 4; Flor's 1, 2, and 6.
	L-2	Rodenhiser and Stakman's 2; Holton's Marquis race; Aamodt's 2; collections from Brookings and Redfield, S. Dak.; Fargo and Dickinson, N. Dak.
	L-3	Gaines' 5; Bressman's 3; Flor's 3 and 5; Melchers' 1 and 5; Young's Montana collection 8; Tingey's Utah collection 2; collections from Bozeman and Moccasin, Mont.
	L-4	Bressman's 4; Tingey's Utah collection 6.
	L-5	Melchers' 2.
	L-6	Collection from Moore, Mont.
	L-7	Flor's 4; Tingey's Utah collection 4.
	L-8	Bressman's 6.

[1] Additional races have been tested that were identified by earlier investigators on different hosts not available for these tests. It may be of interest to record their reaction on the present host testers. Aamodt's races 3 and 4 of *Tilletia levis* had the same reaction as his race 2, reclassified here as L-2. Likewise, Melchers' race 7 was the same as his race 2, reclassified here as L-5.

TABLE 3.—*Relative susceptibility*[1] *of 10 differential hosts to 11 physiologic races of Tilletia tritici and 8 physiologic races of T. levis*

[R=0-10 percent; I=11-40 percent; S=41-100 percent]

TILLETIA TRITICI

Physiologic race no.	Hybrid 128 (C. I. 4512)	Ridit (C. I. 6703)	Oro (C. I. 8220)	Hohenheimer (C. I. 11458)	Hussar (C. I. 4843)	Albit (C. I. 8275)	Ulka (C. I. 11478)	Marquis (C. I. 3641)	Canus (C. I. 11637)	Mindum (C. I. 5296)
T-1	S	R	R	R	R	R	S	[2]**I**	R	**R**
T-2	S	R	R	R	R	R	S	**R**	R	**S**
T-3	S	**R**	R	R	**R**	R	S	S	**S**	I
T-4	S	R	R	R	R	**I**	S	S	**R**	I
T-5	S	R	R	R	R	**I**	S	S	**S**	I
T-6	S	R	R	R	**R**	**S**	S	S	R	I
T-7	S	R	R	R	**I**	**S**	S	S	I	I
T-8	S	R	R	R	**S**	**S**	S	S	S	I
T-9	S	R	R	**I**	R	**R**	S	I	R	I
T-10[1]	S	R	R	**S**	R	**R**	R	I	R	R
T-11	S	**S**	R	R	R	R	I	S	**S**	I

TILLETIA LEVIS

L-1	S	R	R	R	R	R	S	**I**	**R**	I
L-2	S	R	R	R	R	R	S	**S**	**R**	I
L-3	S	R	R	R	R	R	S	**S**	**S**	I
L-4	S	R	R	R	R	**S**	S	I	**R**	I
L-5	S	R	R	R	**R**	**S**	S	S	**S**	I
L-6	S	R	R	R	**I**	**S**	S	S	S	I
L-7	S	R	R	R	**S**	**S**	S	I	S	I
L-8	S	R	**S**	R	R	**R**	S	S	S	I

[1] The reaction of spring wheat differential hosts to this race was obtained in 1936 only, and the results are therefore not strictly comparable with those recorded for the other races. The results are included, however, to indicate particularly the resistance of Ulka to race T-10.

[2] Reactions that differentiate physiologic races are indicated by bold-face type.

The results indicate further that certain varieties, completely susceptible to a large number of races, may carry factors for resistance to others. The spring wheat variety Ulka was originally selected as the susceptible check on which to base the relative susceptibility of the differential hosts. It was found in later tests that Ulka, although completely susceptible to most races, carries factors for resistance to T–10 and T–11. It is possible, then, to strain out certain races from mechanical mixtures even on what has been generally considered a susceptible variety. The races T–10 and T–11 should be noted also with regard to their reaction on the varieties Hohenheimer and Ridit. Although lacking in factors for pathogenicity on Ulka, T–10 is particularly virulent on Hohenheimer, which is highly resistant to all other races except T–9. Hohenheimer is intermediate in its reaction to T–9. Likewise T–11, although relatively low in virulence on Ulka, is extremely pathogenic on Ridit, which is resistant to all other known races.

As indicated in table 2, a number of races which had previously been identified were found to be duplicates. Furthermore, a few smut collections that had been reported as pathogenically distinct did not appear to be so in these tests. Two explanations may be offered for these latter results. Either there were mixtures of races of the same species in the original collections that were later separated by "varietal screening" (*2*, *4*, *17*) or the chlamydospores were of hybrid origin. Usually in making collections 50 or more bunted heads are collected in each field, and within these collections from single fields mixtures of *Tilletia tritici* and *T. levis* are frequently found. This is particularly true of collections made in the western half of the hard red spring wheat area and in the intermountain area of the Pacific Northwest. Pathogenically distinct races also appear to be equally well distributed in these areas, and it would be surprising if some of the original collections did not contain race mixtures. Since the time the inoculum used in the present tests was obtained from the original investigators there has been opportunity for varietal screening of races, for each year inoculum for subsequent tests has been collected from a single differential host. Assuming there were race mixtures in the original collection this would tend to purify the inoculum, and consequently results comparable with those reported originally would not be obtained. Hybridization might also account for the inconsistencies referred to. Relatively little is known regarding the genetics of the bunt fungi; but Flor (*4*) has shown that interspecific and intraspecific hybridization may take place in *Tilletia*, so there is a possibility that the chlamydospores of the original collection were of hybrid origin. Segregates carrying different factors for pathogenicity may have been separated in the process of varietal screening, which would likewise account for the differences in the reactions obtained with the original collection and those in the present tests.

One of the principal objects in standardizing the race numbers and in identifying new races has been to utilize them in the breeding program for the development of smut-resistant varieties of wheat. It is probable that with the present technique for differentiating these races of *Tilletia* minor factors governing pathogenicity are being overlooked.

22709—37——2

When new differential hosts are obtained and when tests are made under controlled environmental conditions not only during the time of infection but also during subsequent plant growth, more of these "border-line" races may be differentiated. However, from the practical point of view, in the development of smut-resistant varieties from the present stock of parental wheats it would seem unnecessary to have to take into account races having these minor differences. For example, from the data recorded in table 1, Oro is shown to be resistant to all races except L–8 while Ridit is resistant to all except T–11. It would be expected, then, that certain progeny from Oro × Ridit would carry factors for resistance to all races. Similarly, as indicated in the data in table 1, resistant progeny should be obtained in crosses of Oro or Ridit with Hussar and Hohenheimer.

Although in this paper emphasis has been placed on the separation of races on the basis of pathogenicity, in studying the inoculum of different collections and the effect on the host plant it was obvious that there were other criteria by which a number of races could be identified. They include differences in the size, shape, and hardness of the smut balls, in the degree of reticulation of the chlamydospore walls, in the color of the chlamydospore mass, in the degree to which certain varieties of wheat are stunted, in the partial smutting of certain varieties, in the degree to which the various races cause dropping of the awns of the variety Ulka and, lastly, in the degree to which they cause laxness in the spikes of infested plants, particularly when grown under greenhouse conditions. Certain of these differences were noticed not only between the races found to be pathogenically different but also between collections which, with the present technique, were found to be pathogenically the same.

REACTION OF VARIETIES UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

Soil-temperature and moisture conditions during the time infection may take place (*11*) and post-infection temperatures (*16*) have been found to be some of the factors determining the amount of smut that may develop in a susceptible variety. Furthermore, Aamodt (*1*) reported that when subjected to different temperatures some physiologic races appeared to respond differently in infection capability. Although the plantings in the present experiments were made at the different stations when soil-temperature and moisture conditions were believed to be optimum for infection, there obviously would be other environmental conditions that would not be constant, such as soil type, fertility, and weather conditions after emergence of the seedlings. The question arises, then, as to the constancy of the reaction of the differential hosts to the physiologic races at different stations and in different years. The average percentages of smut produced by the 11 races of *Tilletia tritici* and the 8 races of *T. levis* at different stations and in different years are recorded in table 1. These data indicate a fairly close correlation in the reaction of each race on all of the winter wheat differential hosts at Pullman, Bozeman, and Kearneysville and in the reaction of three of the four spring wheats at Bozeman and Pullman. There are some exceptions. In 1934, Albit, inoculated with T–4, developed only 13 percent of smut at Bozeman; in 1935, 18 percent at Pullman; and in this same year 44 percent at Kearneysville. Likewise, there was a wide difference in the susceptibility of Hussar to race L–7 at different stations. In 1935 this variety inocu-

lated with L–7 developed 26 percent of smut at Pullman and 81 percent at Kearneysville. It is possible that some varieties may differ in their susceptibility to certain races of *T. tritici* and *T. levis* when grown under different environmental conditions. There is evidence that this may be the explanation for the above-mentioned variations. As indicated in table 1, Mindum was found to be generally more susceptible to certain races at Bozeman than at Pullman. In the same tests, however, the pathogenicity of these races did not vary appreciably on other differential hosts. This would indicate that the variation in susceptibility of Albit, Hussar, and Mindum mentioned above is due to the effect of environment on the host rather than on the fungus. Furthermore, the environmental effect appears to be expressed only in the reaction of certain varieties to certain physiologic races. Additional evidence of this kind is presented in table 4. Turkey (C. I. 6175) was found to be decidedly less susceptible to races T–1, T–4, and T–6 and to L–1, L–2, and L–4 at Kearneysville than at Pullman. The reaction with the other races on this variety were, however, clearly more consistent.

TABLE 4.—*Reaction of Turkey (C. I. 6175) and Hybrid 128 (C. I. 4512) to each of 8 physiologic races of Tilletia tritici and T. levis at Kearneysville, W. Va. and Pullman, Wash., in 1935*

Physiologic race of *T. tritici*	Percentage of smut at localities indicated on—				Physiologic race of *T. levis*	Percentage of smut at localities indicated on—			
	Turkey		Hybrid 128			Turkey		Hybrid 128	
	Kearneysville	Pullman	Kearneysville	Pullman		Kearneysville	Pullman	Kearneysville	Pullman
T–1	7	20	84	82	L–1	5	48	89	92
T–2	25	25	95	81	L–2	16	77	89	92
T–3	84	93	86	92	L–3	72	84	92	89
T–4	13	44	90	93	L–4	10	48	83	96
T–5	87	90	93	91	L–5	71	80	83	88
T–6	5	30	94	91	L–6	74	89	89	93
T–7	72	49	81	90	L–7	91	82	95	85
T–8	87	85	86	95	L–8	85	89	92	95

Obviously, this evidence that certain varieties may differ in response to races of *Tilletia* under different environmental conditions is of significance in connection with the problems discussed in this paper. In order to establish the principle of physiologic specialization within the species or to obtain information with regard to the number of pathogenically distinct races that may be present in a somewhat localized wheat-growing area it would seem justifiable to make tests at a single station. However, to more accurately standardize a general system of classification of races and to determine their significance in a general wheat-improvement program, the virulence of races must be determined in comparative tests made in more than one geographical area.

DISCUSSION AND CONCLUSIONS

Eleven physiologic races of *Tilletia tritici* and eight of *T. levis* have been separated on the basis of differences in their pathogenicity on six varieties of winter wheat and four varieties of spring wheat. Although sufficient data are not yet available to permit a detailed

discussion of the distribution of these races, there is evidence that several of the same races commonly found in the Pacific Northwest are also present in the hard red spring and winter wheat areas. With the interchange of seed and the opportunity for wind dissemination of inoculum it will not be surprising if all the identified races eventually become distributed in all of the wheat-growing areas of the United States. Thus, it follows that the development of a smut-resistant variety of wheat is not a localized problem that may be solved by the development of a variety of wheat resistant to smut collected in the immediate vicinity of the station where the breeding work is done. In order to thoroughly determine the bunt resistance of varieties, the facts emphasize the need of extensive tests conducted in widely separated areas with all of the known physiologic races of the bunt fungi. Obviously, the adoption of a standard system of determining the number and distribution of physiologic races will aid in the progress of this program.

The number of pathogenically distinct races that may be identified from a large number of smut collections depends to a considerable extent on the varieties used as differential hosts and on the limits of the infection classes, which up to the present time have been more or less arbitrarily selected. The races to which numbers have been assigned very probably do not represent the total among the several hundred collections of both species of *Tilletia* that were originally made. In fact, in preliminary tests with recently made smut collections there is evidence of additional races some of which have fairly wide differences in pathogenicity. Furthermore, data were obtained on the pathogenicity of a number of collections not recorded here because of their so-called border-line differences in pathogenicity. However, for the time being, in the program for the development of smut-resistant varieties of wheat, we are particularly interested in those races that have rather wide differences in pathogenicity.

From the results of these experiments it seems that it should be possible to breed varieties of wheat possessing desirable agronomic characteristics and a high degree of resistance to the known races of *Tilletia*. Oro and Ridit are both agronomically desirable wheats and are in commercial production at the present time. Oro is resistant to all of these races except L–8 and Ridit to all except T–11. It would be reasonable to expect certain progeny from Oro × Ridit to carry factors for resistance to all of the known races. Likewise, Hohenheimer and Hussar should be of value as parent stocks in the development of smut-resistant varieties. Hohenheimer, though not a commercially desirable wheat, is susceptible to only one race and intermediate in its reaction to another. Hussar, which is at present being used as a parent for both winter and spring wheat crosses, is susceptible to two races and intermediate in its reaction to two. It seems possible that segregates will be obtained from crosses with some of these varieties in which desirable agronomic characteristics are combined with smut resistance to the known races of *Tilletia*.

SUMMARY

Twenty-four collections of *Tilletia tritici* and 29 of *T. levis*, each of which had been identified as a distinct physiologic race by previous investigators, together with a number of miscellaneous collections of both species, have been tested under comparable conditions at several experiment stations. Under the conditions of these experiments a number of the collections appeared to be duplicates. Eleven physiologic races of *T. tritici* and eight of *T. levis* were pathogenically distinct and have been assigned letters and race numbers T–1 to T–11 and L–1 to L–8, respectively.

The value of using both winter and spring wheat varieties as differential hosts is emphasized. With the varieties used in these experiments certain races may be differentiated on winter wheats alone, some by their reaction on spring wheats, and certain others by their reaction on the combination of both winter and spring varieties.

The spring wheat variety Ulka, although completely susceptible to most races, carries factors for resistance to at least two races. It is possible, then, to strain out certain races from mechanical mixtures even on what is generally considered to be a completely susceptible variety.

A number of physiologic races differed in characteristics other than pathogenicity. They include certain morphological variations in chlamydospores and smut balls and in color of the spore mass and certain differences in their effect on the host plants as regards stunting, dropping of the awns, and degree of laxness in the spikes.

Turkey (C. I. 6175) and Mindum (C. I. 5296) were found to differ in susceptibility to certain races of both species when grown under different environmental conditions. This variation appears to be due to the effect of environment on the host rather than on the fungus and is expressed only in the reaction of certain varieties to certain physiologic races.

There are several agronomically desirable wheat varieties that are highly resistant to a large number of the known physiologic races of *Tilletia tritici* and *T. levis*. No two of these varieties are susceptible to the same race. It should be possible, therefore, to obtain hybrids in which factors governing resistance to all races of *Tilletia* are combined with those governing other desirable agronomic characters.

LITERATURE CITED

(1) AAMODT, O. S.
1931. VARIETAL TRIALS, PHYSIOLOGIC SPECIALIZATION, AND BREEDING SPRING WHEATS FOR RESISTANCE TO TILLETIA TRITICI AND T. LEVIS. Canad. Jour. Research 5: 501–528, illus.

(2) BRESSMAN, E. N.
1931. VARIETAL RESISTANCE, PHYSIOLOGIC SPECIALIZATION, AND INHERITANCE STUDIES IN BUNT OF WHEAT. Oreg. Agr. Expt. Sta. Bull. 281, 44 pp., illus.

(3) FARIS, J. A.
1924. FACTORS INFLUENCING THE INFECTION OF WHEAT BY TILLETIA TRITICI AND TILLETIA LAEVIS. Mycologia 16: 259–282, illus.

(4) FLOR, H. H.
1932. HETEROTHALLISM AND HYBRIDIZATION IN TILLETIA TRITICI AND T. LEVIS. Jour. Agr. Research 44: 49–58.

(5) Flor H. H.
1933. STUDIES ON PHYSIOLOGIC SPECIALIZATION IN TILLETIA TRITICI AND T. LEVIS IN THE PACIFIC NORTHWEST. Jour. Agr. Research 47: 193–213, illus.
(6) Gaines, E. F.
1928. NEW PHYSIOLOGIC FORMS OF TILLETIA LEVIS AND T. TRITICI. Phytopathology 18: 579–588.
(7) ——— and Smith, W. K.
1933. REACTION OF VARIETIES AND HYBRIDS OF WHEAT TO PHYSIOLOGIC FORMS OF BUNT. Jour. Amer. Soc. Agron. 25: 273–284, illus.
(8) Holton, C. S.
1930. A PROBABLE EXPLANATION OF RECENT EPIDEMICS OF BUNT IN DURUM WHEATS. Phytopathology 20: 353–357, illus.
(9) ———
1931. THE RELATION OF PHYSIOLOGIC SPECIALIZATION IN TILLETIA TO RECENT EPIPHYTOTICS OF BUNT IN DURUM AND MARQUIS WHEATS. Phytopathology 21: 687–694, illus.
(10) ———
1935. STUDIES ON SEVEN DIFFERENTIATING CHARACTERISTICS OF TWO PHYSIOLOGIC FORMS OF TILLETIA TRITICI. Phytopathology 25: 1091–1098, illus.
(11) Hungerford, C. W.
1922. THE RELATION OF SOIL MOISTURE AND SOIL TEMPERATURE TO BUNT INFECTION IN WHEAT. Phytopathology 12: [337]–352, illus.
(12) Melchers, L. E.
1934. INVESTIGATIONS ON PHYSIOLOGIC SPECIALIZATION OF TILLETIA LAEVIS IN KANSAS. Phytopathology 24: 1203–1226, illus.
(13) Reed, G. M.
1928. PHYSIOLOGIC RACES OF BUNT OF WHEAT. Amer. Jour. Bot. 15: 157–170.
(14) Rodenhiser, H. A.
1928. PHYSIOLOGIC SPECIALIZATION IN SOME CEREAL SMUTS. Phytopathology 18: 955–1003, illus.
(15) ——— and Stakman, E. C.
1927. PHYSIOLOGIC SPECIALIZATION IN TILLETIA LEVIS AND TILLETIA TRITICI. Phytopathology 17: 247–253, illus.
(16) Smith, W. K.
1932. THE EFFECT OF DIFFERENT TEMPERATURES ON THE REACTION OF HOPE WHEAT TO BUNT. Phytopathology 22: 615–627, illus.
(17) Weston, W. A. R. Dillon.
1929. RESISTANCE OF WHEAT VARIETIES TO BUNT (TILLETIA CARIES). Nature [London] 123: 243.

FUSARIUM RESISTANCE IN WISCONSIN ALL SEASONS CABBAGE [1]

By L. M. BLANK [2]

Assistant pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The control of the yellows disease of cabbage (*Brassica oleracea* L.) has been effected through the development of varieties of cultivated cabbage (*B. oleracea capitata* L.) that are resistant to the soil-borne parasite, *Fusarium conglutinans* Wr. Wisconsin All Seasons, one of the first of the yellows-resistant varieties developed, was released for commercial distribution in 1920 (*6*).[3] The parent strain (XXV–7–2s) was a progeny derived from the self-pollination of a plant of the variety All Seasons which had remained free from disease on yellows-infested soil in the Wisconsin field trial of 1916. Trials of this progeny in the field in 1917 and 1918 gave 5 and 1 percent disease, respectively, while All Seasons showed 80 and 60 percent, respectively. Heads selected from this strain served as the foundation stock from which the resistant variety Wisconsin All Seasons was derived. This variety is widely used for sauerkraut manufacture in the Middle West and consistently shows a high percentage of disease-free individuals on the most severely infested field soils.

Recent studies with Wisconsin Hollander (*1*) have shown that it did not contain the single dominant gene for resistance to yellows, as has been described in a number of the resistant varieties of cultivated cabbage, in brussels sprouts (*Brassica oleracea gemmifera* DC.), in kohlrabi (*B. oleracea caulo-rapa* DC.), and in wild cabbage (*B. oleracea*) (*2, 8, 9, 10, 11*). Resistance to *Fusarium* in Wisconsin Hollander is apparently due to a number of factors, cumulative in effect, which control a type of resistance that is effective at moderate soil temperatures but not at constant soil temperatures of about 24° C. Resistance in the other investigated varieties of cabbage and allied forms is due to a single dominant factor and remains effective at soil temperatures of 24° and slightly above on heavily infested soils. This type of resistance is designated as type A. The other, manifested by Wisconsin Hollander, is designated as type B. The chief difference between the two types, on which the technique of distinguishing them is based, is that type A is due to a single dominant gene, whereas type B is more complex genetically. Plants of type A, when crossed with a very susceptible line, yield F_1 progenies that remain free from yellows when grown on infested soil at a constant

[1] Received for publication Jan. 18, 1937; issued October 1937. Cooperative investigations of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Plant Pathology, University of Wisconsin.

[2] Acknowledgment is gratefully made of valuable suggestions and criticisms by J. C. Walker, professor of plant pathology, University of Wisconsin, and agent, Division of Fruit and Vegetable Crops and Diseases.

[3] Reference is made by number (italic) to Literature Cited, p. 510.

temperature of 24°. Plants of type B so crossed yield progenies that succumb to the disease on infested soil at 24°.

Wisconsin All Seasons has never been examined critically as to the nature of its resistance. The studies herein reported were undertaken to determine the nature of resistance in this variety and to improve the variety in uniformity and in other characters.

MATERIALS AND METHODS

The cabbage material from which selections were made represented two strains of the resistant variety Wisconsin All Seasons (WAS–1 and WAS–2). Other lots used for comparative purposes included two lots of the susceptible variety All Seasons (AS–1 and AS–2), a highly susceptible commercial variety of Danish Ballhead, and a homozygous susceptible line (77– series) derived from a Flat Dutch variety. As the result of numerous field and greenhouse trials, the last two lots of material were known to be very highly susceptible to the yellows disease and apparently carried little or none of type B resistance. Individuals from the 77– line were used as a source of pollen for crosses. Throughout the discussion of results this line will be referred to as a highly susceptible line of cabbage.

The material used for breeding purposes was handled in the manner described by earlier writers (*11*). Controlled pollination was effected in the greenhouse, where flowering branches were covered with glassine bags to reduce the chance of contamination by other pollen. Field trials were conducted on thoroughly infested soil in Racine and Kenosha Counties, Wis. Seedbeds were planted on yellows-free soil, and the seedlings were transplanted to the trial plot during the early part of July. The development of disease symptoms was followed throughout the season, bamboo stakes being used as permanent markers of plants showing external symptoms of the yellows disease.

To supplement the field studies, additional trials were conducted on yellows-infested soil in the greenhouse. Artificially inoculated soil (prepared by adding corn meal-sand cultures of the yellows organism to yellows-free soil), naturally infested soil from Racine County, or a mixture of the two types of soil was used. Thorough infestation of the several soils was evident from the reaction of susceptible seedlings used in each greenhouse experiment. The trials were conducted under semicontrolled and controlled soil temperatures. In the first case, the seedlings were transplanted to benches or flats containing yellows-infested soil and a soil temperature favorable for disease development was attained by adjusting the surrounding air temperature of the house. Wide fluctuations in the soil temperature occurred, but in general a temperature favorable for the expression of disease symptoms was maintained. Controlled soil temperatures were secured by the use of Wisconsin soil-temperature tanks.

EXPERIMENTAL RESULTS

During the early progress of the work it was observed that in the field trials of the progenies from self-pollination there was a marked deficiency of diseased individuals in supposedly homozygous susceptible (*rr*) progenies and in segregating (*Rr*) progenies. It was evident that there exists in Wisconsin All Seasons a condition in some respects

similar to that observed in Wisconsin Hollander by Anderson (*1*) (type B resistance). The inheritance of type A resistance and of type B resistance is discussed separately in the following sections.

TYPE A RESISTANCE

F_1 Progenies

The initial head selections were made from two lots of the Wisconsin All Seasons variety in 1931 and 1932. The heads selected in 1931 represented plants of the best type from a population that had been subjected to severe selection for resistance in a yellows trial conducted in the greenhouse; some of those selected in 1932 were from a yellows-infested field, others from a yellows-free field. The seed grown from the heads selected in 1931 and 1932 was produced under conditions of controlled pollination in the greenhouse in 1932 and 1933, respectively. Some of the blossoms on each plant were self-pollinated and some were crossed with pollen from a highly susceptible line.

The F_1 progenies from self-pollination were tested in the field in 1933, and certain of the lines were retested in the field trials of 1934 and 1935. The progenies of crosses were tested almost entirely in the greenhouse, although several were included in the field trials.

The results of trials in the field, in the greenhouse under semicontrolled soil temperatures, and in the soil-temperature tanks are considered separately (table 1). On the basis of their disease reaction under controlled conditions, the F_1 progenies have been grouped into three classes according to the apparent genotype of the parent plant. The homozygous-susceptible (*rr*) class includes those plants the progenies of which comprise only susceptible individuals. The heterozygous-resistant class (*Rr*) includes those plants the progenies of which, from self-pollination, show approximately 25 percent of the individuals diseased and, from crossing with a homozygous-susceptible line, approximately 50 percent of the individuals diseased. The homozygous-resistant class (*RR*) of plants yielded no significant number of susceptible individuals in the F_1 progeny either from self-pollination or from crossing with a homozygous-susceptible line.

TABLE 1.—*Behavior of F_1 progenies of selected plants showing type A resistance to Fusarium in field and greenhouse*

Conditions of test	Genotype of parent plant	Progenies from self-pollination			Progenies from cross of selected plant with susceptible line		
		Progenies tested	Total plants	Diseased plants	Progenies tested	Total plants	Diseased plants
		Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
Field	*rr*	11	440	27	3	142	54
	Rr	27	2,017	5			
	RR	9	400	.2			
Greenhouse, semicontrolled	*rr*	2	63	92	4	227	98
	Rr	20	2,715	19	34	3,445	46
	RR	2	181	0	9	624	0
Greenhouse, controlled (24° C.)	*rr*	7	218	91	8	643	98
	Rr	21	1,463	22	16	1,152	48
	RR	7	200	0	6	236	0

It soon became evident that the genotypes could not be distinguished on the basis of field trials alone. However, under the more favorable conditions for the disease in the greenhouse trials, both semicontrolled and controlled, the genotypic constitution of plants could be determined quite accurately on the basis of progeny tests.

F_2 Progenies

From 19 of the F_1 selfed progenies selections were made for seed production representing the three genotypes of type A resistance, and F_2 selfed seed was secured from 108 of the F_1 plants. From 103 of the plants seed was obtained from a cross with susceptible plants. The F_2 selfed progenies and the F_1 progenies from the susceptible crosses were tested in the field, some of them during two successive seasons, as well as in the greenhouse. The disease reaction in the greenhouse trials showed that they were derived from mother plants representing one or another of the three genotypes (table 2). Lack of expression of disease symptoms was very evident in certain of the progenies in the field trials, preventing an accurate determination of the number of individuals in the susceptible class. However, when such progenies were tested in the greenhouse this difficulty was largely overcome. Selections from F_1 selfed progenies which had been classified as homozygous for resistance were found to yield completely resistant F_2 families.

Table 2.—*Behavior of F_2 progenies showing type A resistance to Fusarium in field and greenhouse*

Conditions of test	Genotype of parent plant	Progenies from self-pollination [1]			Progenies from cross of selfed F_1 with susceptible line		
		Progenies tested	Total plants	Diseased plants	Progenies tested	Total plants	Diseased plants
		Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
Field	*rr*	18	880	12	7	240	58
	Rr	50	2,641	5			
	RR	39	2,291	0			
Greenhouse, semicontrolled	*rr*	8	746	72	16	1,290	92
	Rr	42	3,128	22	46	2,243	45
	RR	26	1,512	.07	38	1,810	.2
Greenhouse, controlled (24° C.)	*rr*	13	830	89	11	475	99
	Rr	8	355	18	8	480	54
	RR	13	338	0	1	75	0

[1] Selfed 2 generations.

F_3 Progenies

From the selfed F_2 material in the field trials, selections were made chiefly from the homozygous-resistant lines. Inasmuch as resistance had been established in a homozygous form in many of the plant lines, the selections were secured from those lines that appeared to approach most nearly the ideal type of Wisconsin All Seasons. The plants were paired in the greenhouse, sib crosses were made, and on many of the plants a cross with a very susceptible line was also made. The sib-cross progenies were tested for yellows reaction in the field, and all remained free of disease symptoms with the exception of three of the progenies, in each of which one yellows plant was found. Further

study in the greenhouse of these progenies and of the corresponding susceptible-cross progenies revealed that one of the parents in each of the sib crosses was heterozygous for resistance, apparently due to a chance contamination in the F_2 generation with pollen of a susceptible line.

The results presented indicate that a certain percentage of individuals of the Wisconsin All Seasons variety carry the factor for type A resistance. However, it was pointed out that in the initial selections from this variety a large percentage of the individuals carrying only type B resistance were probably eliminated by exposure to high soil temperatures. A majority of the individuals surviving this treatment were either heterozygous or homozygous for the type A gene (*R*). However, certain of the progenies classed as homozygous recessive for this gene (*rr*) on the basis of their greenhouse reaction were highly resistant in the field. In the F_3 selfed progenies having this constitution, only 2 percent of the plants became diseased in the field as contrasted with 93 percent diseased at a soil temperature of 22° to 24° C. in the greenhouse. Resistance in these lines was comparable to that described by Anderson (*1*) in Wisconsin Hollander and is discussed as type B resistance in the following section.

TYPE B RESISTANCE

As pointed out by Anderson (*1*), the expression of type B resistance is most pronounced at the lower soil temperatures on infested soil in field and greenhouse. It was observed that the progenies in Wisconsin All Seasons that were heterozygous or homozygous recessive for type A resistance commonly showed a percentage of diseased individuals lower than the theoretical, particularly in field trials in which commercial susceptible varieties and very susceptible selections from a variety other than Wisconsin All Seasons consistently showed a high incidence of disease. Inasmuch as the presence of type A resistance in any progeny obscures the expression of type B resistance, the studies herein reported were confined to the progenies that were homozygous for the recessive gene, *r*.

In table 3 are presented results of field and greenhouse trials of lines of Wisconsin All Seasons in which type A resistance was lacking. In the field trials the progenies showed a range from all plants diseased (25–67 selfed and 25–117 selfed) to complete freedom from apparent symptoms. Several of the susceptible-cross progenies included in the field trials showed a lower degree of resistance than did the selfed progenies of the female parents of those crosses. When subjected to trials in the greenhouse the progenies gave a much closer approximation to the expected amount of disease than was obtained in the field trials, showing the important influence of soil temperature in the expression of type B resistance. In order to secure additional data concerning the effect of soil temperature on this type of resistance, experiments were conducted in the soil-temperature tanks over a range of soil temperatures. The expression of symptoms increased with rise in soil temperature, not only in percentage of plants diseased but also in the severity of disease manifestation. The influence of soil temperature upon the rate of disease development and severity of expression is illustrated graphically in figure 1.

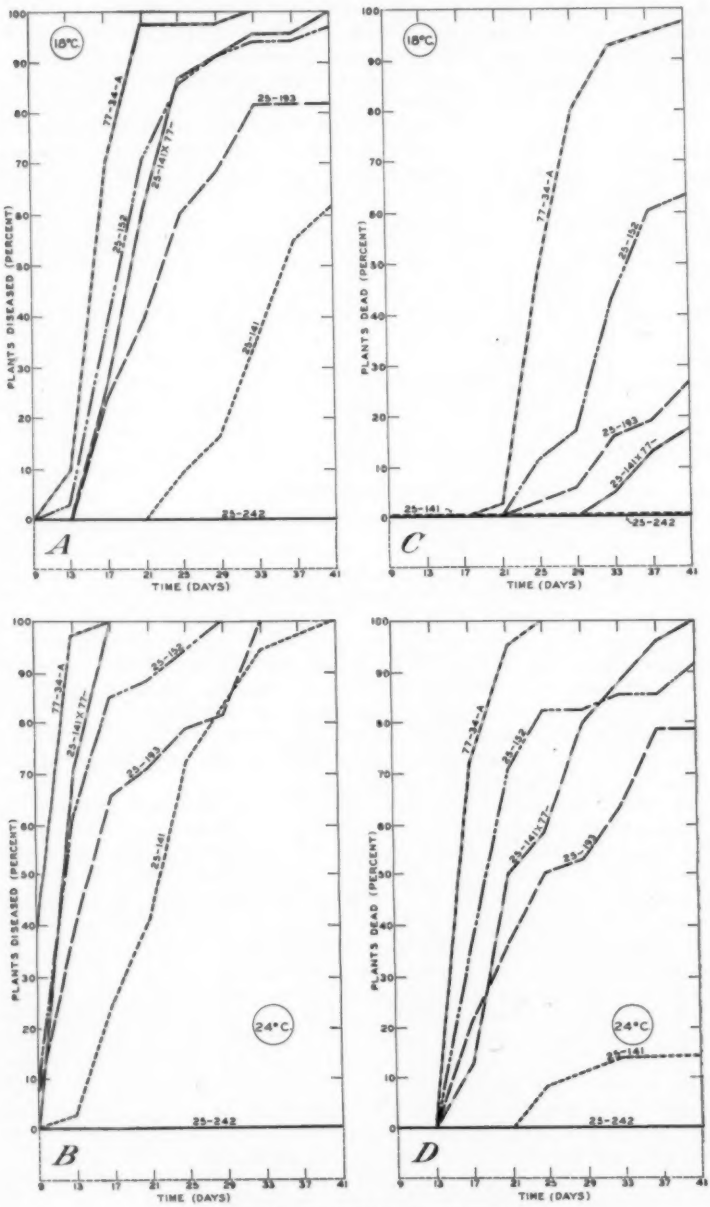

FIGURE 1. (See legend on opposite page)

TABLE 3.—*Occurrence of disease in F_1 progenies of Wisconsin All Seasons cabbage homozygous recessive for type A resistance to Fusarium but carrying various degrees of type B resistance*

Parent plant no.	Progenies from self-pollination of selected plants							Progenies from cross of selected plant with homozygous susceptible line						
	Field trial			Greenhouse, semicontrolled		Greenhouse, controlled (24° C.)		Field trial			Greenhouse, semicontrolled		Greenhouse, controlled (24° C.)	
	Year	Total plants	Diseased plants	Total plants	Diseased plants	Total plants	Diseased plants	Year	Total plants	Diseased plants	Total plants	Diseased plants	Total plants	Diseased plants
		Number	Percent	Number	Percent	Number	Percent		Number	Percent	Number	Percent	Number	Percent
25–28	1933	22	0			33	97	1934	19	0	97	86	126	97
	1934	2	0					1935	37	57				
25–46	1933	52	27			31	94	1934	34	76	20	85	200	99
	1934	9	11											
25–52	1933	9	78			12	100						49	100
25–53	1933	11	0										65	98
25–54	1933	52	65			73	100							
	1934	11	27											
25–58	1933	52	25										77	99
	1934	18	22											
	1935	12	25											
25–60	1933	56	14	26	96	48	67				63	87	33	97
25–67	1933	14	100											
25–68	1933	53	6											
	1934	27	0	37	89			1934	28	32	47	94	69	97
	1935	7	0					1935	24	83				
25–73	1933	12	8			11	91						24	96
25–79	1933	21	57			10	100							
25–117	1933	23	100											
25–136	1934	25	0			45	96				51	100	32	100
25–137	1934	25	0			35	86				35	100		
25–139	1934	17	0			5	80				33	100	59	97
25–141	1934	51	0	6	50	104	80				26	100	24	100
25–152	1934	46	22			114	95				126	90	29	100
	1935	30	80											
25–193	1934	65	18	223	80	185	89	1935	70	94	189	98	99	100
	1935	46	39											
25–205	1934	74	4	30	70	44	93	1935	46	70	124	91	45	98
	1935	30	27											
25–212	1934	44	7	124	62	62	98				139	87	24	100
25–218	1934	68	0	116	57						48	94		
	1935	39	0											

Progeny 25–242 selfed was homozygous for type A resistance and remained free of disease symptoms throughout the trial. Progeny 77–34–A was a very susceptible line, resulting from a cross between several plants that were used as the source of pollen in the susceptible crosses and that were homozygous susceptible for type A resistance. Progeny 77–34–A was practically free from type B resistance as indicated by the rapidity and severity of disease development. Selfed progenies 25–152, 25–193, and 25–141 were homozygous susceptible for type A resistance, but according to field trials (table 3), carried

FIGURE 1.—Comparison of four progenies (25–141 selfed, 25–141 × 77–, 25–152 selfed, and 25–193 selfed) of Wisconsin All Seasons cabbage, showing type B resistance to yellows, with a very susceptible line (77–34–A) and with a progeny (25–242 selfed) homozygous for type A resistance, grown on *Fusarium*-infested soil at constant temperatures of 18° and 24° C. *A* and *B*, Incidence of disease (total affected plants) at 18° and 24°, respectively. The rate of development of symptoms is most rapid at the higher soil temperature. *C* and *D*, Severity of disease (plants dead) at 18° and 24°, respectively. At the lower soil temperature the very susceptible line (77–34–A) succumbed promptly, whereas the progenies carrying type B resistance varied in the rate and severity of disease development. At 24° these progenies succumbed, though less rapidly than did the very susceptible line. The hybrid progeny (25–141 × 77–) between plants high and low in degree of type B resistance was intermediate in its disease reaction between that of the parents. Progeny 25–242 selfed was homozygous for type A resistance and remained free of disease symptoms throughout the duration of the experiment.

different degrees of type B resistance. At both 18° and 24° C., these lines showed more resistance than did 77–34–A and maintained the same relative position as to degree of resistance that they had in the field trials. At 18°, 25–141 selfed showed but 61 percent of the plants diseased and none dead, whereas at 24° all plants were diseased and 14 percent were dead at the conclusion of the experiment. The susceptible-cross progeny of plant 25–141 gave a disease reaction intermediate between that of 25–141 selfed and 77–34–A, substantiating the evidence that, to some extent, type B resistance was transmitted to

FIGURE 2.—Comparison of three lots of cabbage showing varying degrees of type B resistance; trial on naturally infested soil in Kenosha County, Wis., in 1935. *A*, Progeny 25–425s, F_2 inbred generation, remained free of apparent symptoms. *B*, Progeny 25–462 × 77–, a cross of an F_2 plant with a very susceptible line, showed 17 plants diseased in a population of 50; of the diseased plants, 16 were only slightly affected and 1 was dead. *C*, A commercial susceptible line of the Danish Ballhead type; practically all of the plants were diseased, with the majority either severely diseased or dead. The same progenies at a constant soil temperature in the greenhouse are shown in figure 3.

and expressed in the F_1 hybrid of a cross between lines high and low in expression of this character.

From certain of the lines with type B resistance, individuals were selected in the 1933 field trials that had failed to show symptoms. The F_2 inbreds remained, with one exception, entirely free from symptoms in the field (fig. 2), and displayed a degree of type B resistance equal to or exceeding that of their parental lines (table 4). This result was anticipated, inasmuch as the F_2 inbreds were secured from F_1 plants that had remained free from disease in the field and that presumably had carried more type B resistance than did sister plants that had manifested symptoms. In the field, progenies from the crosses with susceptibles showed disease ranging from 5 to 73 percent. The behavior in the field of the F_2 progenies from self-pollination and the progenies of susceptible crosses indicated that type B resistance was dispersed to some extent when a cross was made with plants carrying little or none of this resistance. In the greenhouse the resistance of the F_2 inbreds was broken down (fig. 3).

TABLE 4.—*Occurrence of yellows in F_2 and F_3 progenies selected from plants free from type A resistance to Fusarium but exhibiting type B resistance*

Parent progeny no.	Generation and progeny no.	Progeny from self-pollination							Progeny from cross of inbred F_1 or F_2 with a homozygous susceptible line						
		Field trial			Greenhouse, semicontrolled		Greenhouse, controlled (24° C.)		Field trial			Greenhouse, semicontrolled		Greenhouse, controlled (24° C.)	
		Year	Total plants	Diseased plants	Total plants	Diseased plants	Total plants	Diseased plants	Year	Total plants	Diseased plants	Total plants	Diseased plants	Total plants	Diseased plants
	F_2		*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*		*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*
25–58s	25–262	1934	57	0	10	80	48	56	1935	26	73	37	100	43	100
		1935	19	0											
	25–265	1934	16	0			36	86				72	75	29	100
		1935	2	0											
25–68s	25–275	1934	53	0					1935	26	23	37	86		
		1935	38	0											
	25–423	1935	26	0	99	79	43	94	1935	26	31	158	96		
	25–424	1935	14	0					1935	25	24	127	88	50	100
	25–425	1935	36	0	138	74	61	95							
25–46s	25–411	1935	22	9			48	100	1935	21	5	55	95	41	100
	25–412	1935	14	0								33	73		
	F_3														
25–262s	25–461				158	88	85	87				53	96		
	25–462	1935	74	3	95	84	60	93	1935	50	34	175	80	59	86
25–265s	25–465	1935	4	0											
25–275s	25–468	1935	16	0								10	80		
	25–472	1935	19	0											

Several of the F_2 progenies were carried through another generation. Although the number of progenies involved was small, there was evidence that type B resistance was relatively stable through three generations of self-pollination (fig. 4). As usual in greenhouse trials, type B resistance was broken down upon exposure of seedlings to higher soil temperatures.

TABLE 5.—*Occurrence of disease in F_2 progenies of a cross between plants carrying type B resistance and a very susceptible plant*

Cross no.	F_1 progeny no.	F_2 progenies from self-pollination							Progeny from backcross with homozygous susceptible line						
		Field trial			Greenhouse, semicontrolled		Greenhouse, controlled (24° C.)		Field trial			Greenhouse, semicontrolled		Greenhouse, controlled (24° C.)	
		Year	Total plants	Diseased plants	Total plants	Diseased plants	Total plants	Diseased plants	Year	Total plants	Diseased plants	Total plants	Diseased plants	Total plants	Diseased plants
			No.	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*		*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*
25–28 × 77–	25–401	1935	26	54											
	25–402	1935	30	37											
	25–403	1935	26	73			45	100	1935	38	87			45	100
	25–404	1935	30	33			35	100	1935	28	93				
	25–405	1935	25	8			27	89							
25–46 × 77–	25–415	1935	43	93	107	100	47	100				173	100	49	100
	25–417	1935	34	88	42	100	52	100	1935	54	61	169	93	49	100
	25–418	1935	41	49	71	97	55	98	1935	41	39	73	99	54	100
	25–419	1935	25	72			7	100				150	97		
	25–420	1935	33	64								100	99		
25–68 × 77–	25–426	1935	34	71	101	99	62	100	1935	27	93	180	99	50	100
	25–429	1935	38	82	56	100	60	100	1935	41	100	92	97	56	100

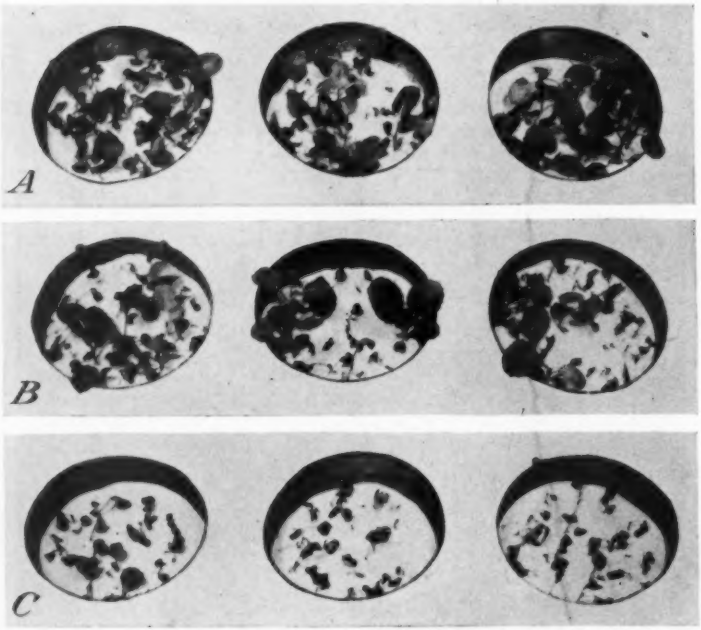

FIGURE 3 (See legend on opposite page)

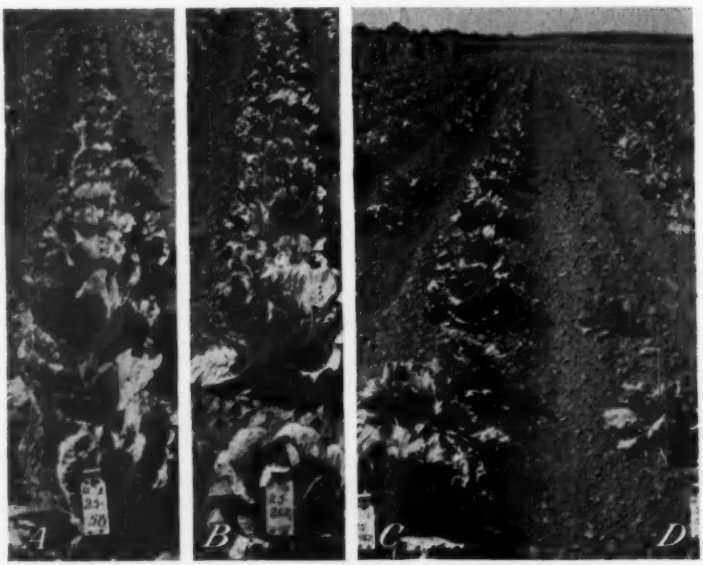

FIGURE 4 (See legend on opposite page)

From the progenies of crosses of plants 25–28, 25–46, and 25–68 with susceptibles, selections were made of those progenies that showed in the 1934 field trial 0, 76, and 32 percent plants diseased, respectively (table 3). Seed was produced from the selected heads, and the results of the resistance tests are presented in table 5.

DISCUSSION

The object of this investigation was to determine the nature and inheritance of resistance to *Fusarium* in the Wisconsin All Seasons variety of cabbage. The presence of type A resistance, due to a single dominant gene, was demonstrated in inbred progenies from many of the plant selections. This was in accord with the results of other workers on other varieties of cabbage and certain of the subspecies of *Brassica oleracea*. However, it was found that in Wisconsin All Seasons there was also a second type of resistance, referred to as type B. The latter type is apparently similar to that described by Anderson for Wisconsin Hollander, in which type A resistance is lacking. Type B resistance is characterized by an optimum manifestation at the lower soil temperatures, but at the higher soil temperatures it breaks down and the plants succumb to the disease. This response to soil temperatures made it impossible to differentiate accurately between the two types of resistance in field trials and necessitated trials under conditions of higher soil temperatures in the greenhouse.

Inasmuch as the presence of type A resistance, either in the homozygous dominant (*RR*) or heterozygous (*Rr*) form, obscured the manifestation of the presence of type B resistance, it was necessary to work with progenies that were homozygous recessive (*rr*) for type A resistance. Such progenies varied greatly in the degree of type B resistance, showing a range in the 1933 field trials from all plants diseased in two of the progenies to complete freedom from symptoms in other progenies. Selections from F_1 progenies, which apparently carried a considerable degree of type B resistance, yielded F_2 and F_3 progenies that maintained a high degree of such resistance in succeeding field trials. Nevertheless, the resistance of all was capable of being broken down under greenhouse trials, where more severe conditions prevailed. It was observed, however, that the progenies tested in the greenhouse displayed the same relative degree of type B resistance as was shown in the field trials. The presence of type B resistance was manifested in greenhouse trials by the delayed development of symptoms and their less serious expression. In some of the progenies showing a high degree of type B resistance it was only by prolonged exposure at the higher soil temperatures that a complete or nearly complete break-down of resistance could be secured.

FIGURE 3.—Comparison of the three lots of cabbage shown in field trials in figure 2, when grown on infested soil in the greenhouse at a controlled soil temperature of 23° C.; photographed 19 days after being transplanted. *A*, Progeny 25–425s: 77 percent diseased and 28 percent dead. *B*, Progeny 25–462 × 77–, a susceptible cross of an F_2 plant: 79 percent diseased and 63 percent dead. *C*, A commercial susceptible line of the Danish Ballhead type: 100 percent diseased and 88 percent dead. The relative resistance shown in the field trials was maintained; but the infection conditions were more severe, and all plants finally succumbed to the disease.

FIGURE 4.—Comparison of two lines of cabbage, both homozygous for the recessive gene (*r*) for type A resistance but differing in the degree of type B resistance. *D*, Line 77–, in which there is little or no type B resistance. *A*, *B*, and *C*, The F_1, F_2, and F_3 progenies (25–58, 25–262, and 25–462), respectively, of a line in which a high degree of type B resistance occurred. In the field trials there were 25, 0, and 3 percent plants diseased in the F_1, F_2, and F_3 progenies, respectively, but these progenies showed close to 100 percent of plants susceptible at a constant soil temperature of 24° C. (tables 3 and 4).

In lines of cabbage carrying both type A and type B resistance, the latter type is distinguishable only in those individuals that may segregate as homozygous recessives for type A resistance. That type B resistance is of importance is evident in the behavior of the resistant variety Wisconsin All Seasons. On thoroughly infested soil in Wisconsin this variety seldom shows as much as 10 percent disease; whereas, when tested at high soil temperatures (24° C.) in the greenhouse, it may show as high as 30 percent disease. Under field conditions there are apparently a considerable number of homozygous recessives for type A resistance that contain a sufficiently high degree of type B resistance to enable them to remain free of apparent disease symptoms. Wisconsin Hollander, a variety of cabbage that carries only type B resistance, illustrates the effective value of the latter in seasons when temperatures are moderate. Conversely the ineffectiveness of type B resistance is very evident in Wisconsin Hollander during seasons of high soil temperatures, when a major part of the crop often becomes diseased.

Evidence of the presence of what we now refer to as type B resistance may be had by an examination of earlier studies on inheritance of resistance to *Fusarium* in cultivated cabbage and in other subspecies of *Brassica oleracea*. Walker (*8*) earlier pointed out that differences in the rate and severity of disease development occurred among various susceptible progenies in certain cabbage varieties and that in addition to the main gene for resistance there might be hereditary factors which would modify the expression of the disease. In the light of present knowledge it is reasonable to believe that many of the plant lines which displayed a considerable number of plants in the "mildly susceptible" or "recovered" classes were expressing type B resistance.

The extent to which type B resistance can be concentrated in any given line of cabbage is problematic. The results of present studies indicate that a high degree of type B resistance was maintained through three generations by self-pollination.

The inheritance of resistance to other vascular Fusaria has been studied in the pea (*Pisum sativum* L.), in flax (*Linum usitatissimum* L.), and in cotton (*Gossypium* spp.). Wade (*7*) working with a number of varieties of canning peas, found that resistance to fusarium wilt (*Fusarium orthoceras* var. *pisi* Linford) was based on a single dominant gene. There is very little suggestion in his results of a condition similar to that due to type B resistance in cabbage. As a result of studies on the inheritance of resistance in flax to wilt (*F. lini* Bolley) Burnham (*3, pp. 738, 748*) said:

> The fact that there are strains that apparently breed true for different degrees of wilting may be taken as evidence that several factors are concerned in wilt resistance. One might assume different genetic complexes for the various degrees of resistance; and, further, that each has its own normal curve of variation in development of the resistant property. Under different environments different proportions of the least resistant side of the curve would succumb. The occurrence of some wilt in resistant lines which probably are homozygous, makes genetic analysis of the crosses more difficult. * * * Crosses between certain resistant lines of different origin showed a high percentage of wilt, indicating that they may carry different factors for resistance.

Burnham (*3*) found in general that flax families showing high or low percentages of wilt in the field gave the same reaction in the greenhouse, which is similar to the behavior of cabbage lines with type B resistance.

Studying the inheritance of resistance to fusarium wilt (*Fusarium vasinfectum* var. *aegyptiacum* Fahmy) in a number of varieties of Egyptian cotton, Fahmy (*5*) concluded that resistance and susceptibility are hereditary characters, immunity being dominant and appearing to give a simple Mendelian segregation. In crosses between immune plants and highly susceptible ones, he found in the F_2 generation three immune to one susceptible when plants that showed mild symptoms and recovered, as well as plants that succumbed, were considered as susceptible. The class that Fahmy designated as resistant included plants which showed typical leaf mottling in the seedling stage but which recovered and developed into apparently normal plants. In the adult stage such plants commonly showed a restricted darkening of the vascular cylinder of the root, from which the causal organism of wilt could be isolated. Differences were noted by Fahmy in the rate of development of symptoms in the different lines studied. It is probable that in the material studied by him there were modifying factors for resistance, similar to type B resistance in Wisconsin All Seasons cabbage, as well as resistance controlled by a single dominant gene.

Edgerton and Moreland (*4*) made no attempt to determine the inheritance of resistance to *Fusarium* in the tomato, but their results indicate that in the material they observed resistance is probably of the intermediate type, similar to type B resistance in cabbage. It is of significance to note that, while the percentage of plants showing evident (external) symptoms of wilt in the wilt-resistant tomato varieties was rather low, the actual percentage of wilt-infected plants, as determined by the cutting of stems, was much higher. A similar condition has been noted in cabbage plants of susceptible progenies which may manifest no apparent symptoms throughout the growing season but which, on examination of cut stems or roots, may show evidence of infection.

SUMMARY

This paper reports a study of two types of resistance of the Wisconsin All Seasons variety of cabbage to the yellows organism, *Fusarium conglutinans* Wr. Plants were selected from a population that had undergone a severe reduction in numbers from yellows as well as from a population grown on yellows-free soil. The plants were self-pollinated and crossed with homozygous-susceptible plants from a highly susceptible line.

The progenies were tested for yellows reaction on thoroughly infested soil in the field and in the greenhouse. The greenhouse trials were conducted with semicontrol and control of soil temperatures.

The Wisconsin All Seasons variety carries at least two types of resistance. Type A resistance, due to a single dominant gene, is apparently similar to that described for other varieties of cabbage, for brussels sprouts, and for kohlrabi. It remains effective at relatively high soil temperatures (about 24° C.). Type A resistance was demonstrated in progenies of many of the plant selections. It was transmitted through the three generations of material studied. A second type of resistance, referred to as type B, was also found in Wisconsin All Seasons. It is apparently similar to that described for Wisconsin Hollander, and is complex genetically, apparently influenced by several genetic factors. It is characterized by having its most effective expression at the lower soil temperatures, while at the

higher soil temperatures of about 24° plants that have only type B resistance succumb to the disease.

Under the conditions prevailing in the field trials, type B resistance was effective in different degrees in the several progenies. Some, evidently carrying high degrees of such resistance, showed no external symptoms of the disease; other progenies gave evidence of less resistance by development of a considerable percentage of mild infection; and still others succumbed promptly to the disease.

In the greenhouse trials with controlled soil temperatures the expression of type B resistance was suppressed, all progenies eventually succumbing to the disease. Under semicontrolled or fluctuating temperatures, type B resistance was partially expressed, resulting in a delayed and less severe development of symptoms of the disease.

In the greenhouse trials, as in the field, different progenies showed differences in rate and severity of disease development, indicating various degrees of type B resistance.

The severity of yellows disease in Wisconsin All Seasons cabbage depends on the genetic make-up with reference to type A and to type B resistance, on soil temperature, and possibly on other conditions that influence the expression of resistance.

LITERATURE CITED

(1) Anderson, M. E.
1933. Fusarium resistance in Wisconsin Hollander cabbage. Jour. Agr. Research 47: 639–661, illus.

(2) Blank, L. M., and Walker, J. C.
1933. Inheritance of fusarium resistance in Brussels sprouts and kohlrabi. Jour. Agr. Research 46: 1015–1022.

(3) Burnham, C. R.
1932. The inheritance of fusarium wilt resistance in flax. Jour. Amer. Soc. Agron. 24: 734–748.

(4) Edgerton, C. W., and Moreland, C. C.
1921. Tests of the wilt resistance of different tomato varieties. La. Agr. Expt. Sta. Bull. 184, 24 pp., illus.

(5) Fahmy, T.
1931. The genetics of resistance to the wilt disease of cotton and its importance in selection. Egypt Min. Agr. Tech. and Sci. Serv. Bull. 95, 30 pp., illus.

(6) Jones, L. R., Walker, J. C., and Tisdale, W. B.
1920. Fusarium resistant cabbage. Wis. Agr. Expt. Sta. Research Bull. 48, 34 pp., illus.

(7) Wade, B. L.
1929. Inheritance of fusarium wilt resistance in canning peas. Wis. Agr. Expt. Sta. Research Bull. 97, 32 pp., illus.

(8) Walker, J. C.
1930. Inheritance of fusarium resistance in cabbage. Jour. Agr. Research 40: 721–745, illus.

(9) ———
1933. Yellows-resistant lines of early Jersey Wakefield cabbage. Jour. Agr. Research 46: 639–648, illus.

(10) ——— and Blank, L. M.
1934. Fusarium-resistant Danish ballhead cabbage. Jour. Agr. Research 49: 983–989, illus.

(11) ——— Monteith, J., Jr., and Wellman, F. L.
1927. Development of three midseason varieties of cabbage resistant to yellows (Fusarium conglutinans Woll.). Jour. Agr. Research 35: 785–809, illus.

FACTORS AFFECTING CHLOROSIS IN IRRIGATED WHEAT [1]

By D. W. ROBERTSON, *agronomist*, and ROBERT GARDNER, *associate agronomist*, *Colorado Agricultural Experiment Station*

INTRODUCTION

A tendency of Marquis wheat to turn pale when irrigated at germination has been noticed in previous studies, and a similar tendency has been observed in the field when heavy rains occurred soon after the wheat emerged. The pale-green color may last for 3 to 6 weeks before the plants recover their normal green appearance. This mild chlorotic condition has been attributed to the use of cold water for irrigation, but experimental data to support this belief are lacking. Because of this a study was made of the effect on chlorosis of (1) the temperature of the irrigation water at germination and (2) the type of fertilizer used.

EFFECT OF TEMPERATURE OF IRRIGATION WATER ON YIELD OF WHEAT IRRIGATED AT GERMINATION

EXPERIMENTAL METHODS

Studies on the effect of applying irrigation water of different temperatures, to Marquis wheat (*Triticum aestivum* L.) on Fort Collins loam (*7*) [2] were conducted for a 4-year period from 1927 to 1930, inclusive.

The experiment was carried on each year on land which had been summer-fallowed the previous year. This summer fallow was necessary in order to obtain a more uniform moisture content in the soil and also to eliminate volunteer grain and control weeds. In 1928 and 1929, a single series of 10 plots, each one five-hundredths of an acre in size, was used. In 1930, two series of similar plots were employed. Each series was planted to Marquis wheat at the rate of 90 pounds per acre. One drill width of 16 rows was planted in each series.

The wheat was planted on April 8 in 1929 and April 7 in 1930. Canvas covers similar to those described by Robertson et al. (*5*) were used to eliminate the effects of rainfall in 1929 and 1930. Water was applied at the rate of 6 acre-inches [3] to each plot at germination (*5*). The temperatures of the irrigation water applied in 1928 were 41° and 62° F., and in 1929 and 1930 they were 40° and 60°.

The basin method of irrigation was used throughout the investigation. The plots were diked and sufficient water was applied to give the required depth. The amount of water required for a given depth was measured for each plot. The temperatures were controlled by ice or steam, depending on whether the water in the pipe line was higher or lower than the temperature required. The source of the irrigation water is discussed in a previous publication (*5*).

[1] Received for publication Apr. 1, 1937; issued October 1937.
[2] Reference is made by number (italic) to Literature Cited, p. 520.
[3] Inches of irrigation water refer to inches in depth over the area.

The area of each plot harvested was one eight-hundred-and-seventy-firsts of an acre. The 10 center rows were harvested after 3 border rows on each side and 1 foot from each end had been discarded to eliminate possible border effect. The grain was cut 1 inch above the ground with lawn shears, tied in a sheaf, and carefully wrapped in cloth to protect the heads, leafy material, and straw. These sheaves were shocked under cover and allowed to cure for 3 weeks or more. The sheaves were then weighed and the grain threshed. The difference between the cleaned grain weight and the total grain and straw weight was used as the straw yield.

EXPERIMENTAL RESULTS

The studies conducted with irrigation water at temperatures of 40° and 60° F. applied at germination, show a slight difference in grain yield in favor of the plots receiving the water at 40°. However, in 1930 when the number of replications was sufficient to make the application of statistical methods possible, no significant differences were obtained. The straw yield was slightly higher for the plots irrigated with water at 60°, but was not significantly different (table 1).

TABLE 1.—*Yields of grain and of straw from plots irrigated at germination with water at 40° or 60° F.*

Year grown	Plots	Yield per plot when irrigation water at indicated temperature was used			
		40° F.		60° F.	
		Grain	Straw	Grain	Straw
	Number	*Grams*	*Grams*	*Grams*	*Grams*
1929	2	398	820	373	837
1930	4	330±12	617±26	318±12	656±26

Standard error obtained by the analysis of variance (2).

An examination of the soil-temperature curves in figure 1, *A* (1928), shows that there was a rise in temperature, in the first foot of soil, of about 4° F. 24 hours after treatment. The second day after irrigation, the temperature dropped to within 1.5° of the low-temperature treatment. After the fourth day the difference between the two treatments did not exceed 2°.

In the second foot (fig. 1, *B*, (1928) there was a difference of 5.5° F. between the two treatments 24 hours after the water was applied. The temperature had become equal after 3 days and fluctuated only slightly thereafter.

In 1929 (fig. 2, *A*,) the temperatures of the soil in the plots came within 0.5° F. of each other 3 days after treatment, and did not fluctuate beyond 1.5° during the 10-day period after irrigation. In the second foot (fig. 2, *B*), there was very little difference.

These results, considered in connection with the yield data, indicate that a difference in temperature in the irrigation water of 20° F., i. e., between 40° and 60°, has no effect on the yield of Marquis wheat irrigated at germination.

The minimum temperature of the air is plotted in figures 1 and 2, along with the soil temperatures. It will be noted that the soil

temperature seems to be influenced more by the fluctuation of the air temperature than by the temperature of the irrigation water. This is especially noticeable in the curves for temperature 4 days after the application of the irrigation water. The following theoretical discussion indicates that a rise in temperature of about 10° F. should be the maximum difference expected if outside influences have no effect on the soil complex and the water added is the only contributing factor to the temperature change.

If water and soil of different temperatures are mixed, the final temperature of the complex (assuming no energy changes due to chemical reactions) may be expressed by the equation:

$$\frac{c_1 m_1}{c_2 m_2} = \frac{^\circ F_2 - X}{X - ^\circ F_1} \qquad (1)$$

where c_1=specific heat[4], m_1=the mass, and $^\circ F_1$=the temperature of the constituent having the lower temperature, and c_2, m_2, and $^\circ F_2$=the corresponding properties of the constituent of higher temperature, and X=the final temperature.

If water is already present as soil moisture before irrigation, it might be expected that the heat capacity of the soil plus moisture would determine the final temperature. However, when water is added to the soil surface it does not mix intimately with the soil moisture already present, but to a large extent replaces it as the portion already present moves downward. It is a question as to how much exchange of heat there is between moisture in the soil and moisture added under these conditions. Furthermore, the upper layers of soil are changed a little by each succeeding increment of water which enters, so that at the completion of irrigation the surface will approach the temperature of the water added and the extreme depth of penetration will approach the temperature before irrigation. The problem is thus complicated by an unknown degree of mixing with the soil moisture and the development of a temperature gradient. Other complications, of course, are the effects of evaporation and heat radiation during the process.

If we assume that the water added warms or cools the soil only and not the moisture already present and that the evaporation and radiation effects may be neglected, the following example shows the average final temperature and depth of temperature change which should be expected from adding 6 inches of water at 60° F. to a soil at 40°:

Average field capacity of upper 2 feet (estimated)	18.0 percent.
Ratio of weight of soil to weight of water	5.55/1.

Substituting in equation 1—

$$\frac{0.2 \cdot 5.55}{1} = \frac{60 - X}{X - 40} = 49.47^\circ \text{ F.}$$

Weight of water applied per square foot of surface	31.25 pounds.
Average weight per cubic foot of soil (determined)	88.3 pounds.
Weight of water absorbed per cubic foot (18 percent of soil)	15.89 pounds.
Depth of water penetration and of temperature change (no. 3÷ no. 5)	1.96 feet.

[4] Approximate specific heat of soil obtained from Patten (4).

The result is approximately a maximum average rise of 10° F. in soil temperature to a depth of 1.96 feet. The graphs show, as could be expected, that the actual rise in the field was much less than this.

The change in temperature, while appreciable, is small compared with the change which could be expected from a snowstorm. Since the heat of fusion of ice is 79.6 calories, an inch of water as ice would cool an equivalent amount of water nearly 144° F.

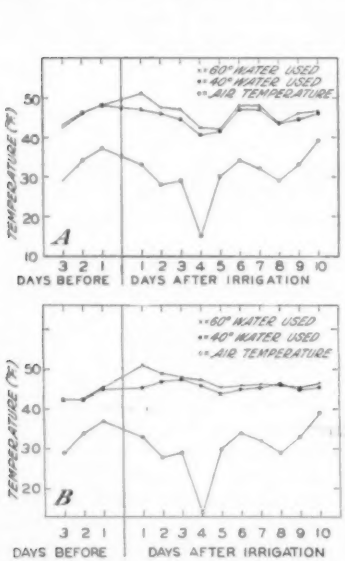

FIGURE 1.—Temperature of the first (*A*) and second (*B*) foot of soil for a 3-day period immediately before and a 10-day period immediately after irrigation with water at 40° and at 60° F., as compared with the air temperature, 1928.

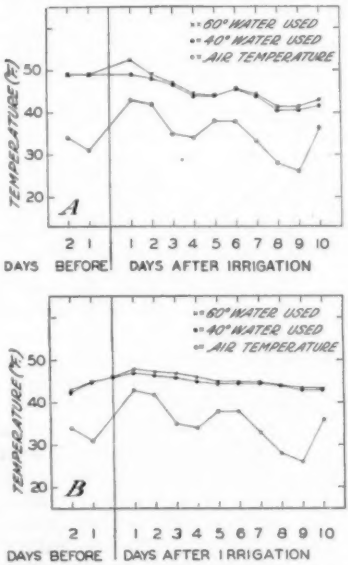

FIGURE 2.—Temperature of the first (*A*) and second (*B*) foot of soil for a 2-day period immediately before and a 10-day period immediately after irrigation with water at 40° and at 60° F., as compared with the air temperature, 1929.

EFFECT OF DIFFERENT FERTILIZER TREATMENTS AT GERMINATION ON THE YIELD OF WHEAT AND ON THE NITROGEN CONTENT OF SOIL, GRAIN, AND STRAW

EXPERIMENTAL METHODS

In 1933 a study was started to determine the effect of different fertilizer treatments at germination on the development of chlorosis and the recovery of Marquis wheat showing chlorosis. Uncovered plots of similar size to those used in the temperature study were employed. The plots were laid out in a 7 by 7 Latin square. The chemical treatments and the amounts used are given in table 2.

TABLE 2.—*Chemical treatments and amounts applied per acre*

Treatment	Salt used	Quantity per acre
		Pounds
Manganese sulphate	$MnSO_4+4H_2O$	318.70
Iron sulphate	$FeSO_4+7H_2O$	297.22
Superphosphate	$CaH_4(PO_4)_2+H_2O$	360.25
Ammonium-acid phosphate	$(NH_4)H_2PO_4$	328.80
Ammonium sulphate	$(NH_4)_2SO_4$	188.77
Calcium nitrate	$Ca(NO_3)_2+4H_2O$	337.35

Marquis wheat was planted on April 3, 1933, April 9, 1934, and April 1, 1935.

Treatment of plots.—In 1933 irrigation water was applied at the rate of 6 inches when the wheat had germinated and was just emerging. The various series were irrigated as follows: April 20, series E; April 24, series F and G; April 25, series H, I and plots 3, 4, 5, and 6 on Series J; April 26, series K and plots 7, 8, and 9 on series J. The chemicals were dissolved in water and sprinkled on the plots within 24 hours after the 6-inch irrigation. After treatment with the chemical, 1 inch of water was applied to each plot.

In 1934 a 6-inch irrigation was applied on April 20 to series A and B; on April 21 to series C; on April 23 to series D and E; and on April 24 to series F and G. The treatments were applied in a manner similar to that used in 1933. On June 20 and 21, an additional 3 inches was applied to each of the plots.

In 1935 a 4-inch irrigation was applied on April 5 to series A, B, and C. A similar irrigation was applied on April 6 to series D, E, and F. On April 8, series G was irrigated with 4 inches of water. This irrigation was necessary because of the dry season of 1934 and of the months of January, February, and March in 1935. On April 20, when the wheat had germinated, an additional 5-inch irrigation was applied to series A and B. On April 22, series C and D were irrigated. Series E, F, and G were irrigated on April 23. The chemicals were applied on all plots in series A to F on April 23 and to series G on April 24. Sufficient rain fell on April 24 and 25 to carry the fertilizers into the soil so no additional irrigation water was applied.

Statistical analysis.—In the interpretation of the data from the crop-and-soils tests, the analysis of variance *(2)* was used. The data were so arranged that the various interactions could be tested. Table 3 presents the analysis of variance for the yield of grain in grams per plot.

TABLE 3.—*Analysis of variance for yield of grain per plot for the 3-year period, 1933 to 1935, inclusive*

Variance due to—	Degrees of freedom	Sum of squares	Mean square	σ	F [1] *(6)*
Years	2	763,069	381,535	.....	180.65
Rows and columns within years	36	409,889	11,386	.....	5.39
Treatments	6	224,423	37,404	.....	17.71
Treatments x years	12	122,180	10,182	.....	4.82
Error	90	190,127	2,112	45.96	.....
Total	146	1,709,688	.....	.....	.....

[1] All higher than 1-percent point.

The F test indicates that there is a significant difference in the yields between years, rows, and columns within years, and treatments × years; the last-named difference shows that the treatments reacted differently in different years.

The test further shows that response of Marquis wheat to some of the fertilizer treatments is significant. Similar results were obtained for the straw yields and for the total yield of straw and grain combined.

In 1933 the effect of the various treatments first became noticeable about May 30. The plots treated with calcium nitrate were a much darker green than the other plots. The plots receiving ammonium fertilizer were a shade lighter, but could be distinguished from the other fertilizer and the no-treatment plots, which were light green in color. In 1934 and 1935, a similar condition was noticeable.

EXPERIMENTAL RESULTS

Yield of Grain and Straw

The average yields in grams per plot for the 3-year period are given in table 4.

TABLE 4.—*The 3-year average yield of grain and of straw from plots irrigated at germination and later treated with different fertilizers*

Treatment	Yield per plot			Treatment	Yield per plot		
	Grain	Straw	Total		Grain	Straw	Total
	Grams	*Grams*	*Grams*		*Grams*	*Grams*	*Grams*
$FeSO_4$	832	1,179	2,011	$(NH_4)_2SO_4$	886	1,319	2,205
$CaH_4(PO_4)_2$	838	1,189	2,027	$NH_4H_2PO_4$	911	1,349	2,260
$MnSO_4$	840	1,191	2,031	$Ca(NO_3)_2$	939	1,492	2,431
No treatment	844	1,202	2,046				
				Level of significance (2 S.E. (difference)	28	49	77

The grain yields of the plots treated with ammonium sulphate, ammonium phosphate, and calcium nitrate are significantly higher than the yields of the no-treatment plots. The yields of plots treated with iron sulphate, superphosphate, or manganese sulphate do not differ significantly from the check. The straw yields show the same trend. The ammonium- and nitrate-treated plots gave the highest yields. These data indicate that the chlorotic condition of Marquis wheat produced by early irrigations evidently is due to a nitrogen deficiency.

To study further the effect of early irrigations on Marquis wheat, soil samples were taken at intervals throughout the season and analyzed for nitrate nitrogen. Samples were taken in all plots, making, in all, seven samples for each treatment. The samples were taken for the following depths of soil: 0–6, 6–12, 12–24, and 24–36 inches. The nitrate nitrogen was determined by the method described by Gardner and Robertson (*3*), p. 5.

Nitrate Nitrogen Content of Soil

Soil samples were taken in all plots after the irrigation and chemical treatments had been applied. The samples were taken on the following dates: April 29, 1933; April 27, 1934; and May 1, 1935. Different

composite samples were drawn for the different tests. The nitrate nitrogen is recorded in parts per million as nitrogen. Table 5 gives the average nitrogen content of the soil for different depths taken in the spring of 1933, 1934, and 1935.

TABLE 5.—*Average quantity of nitrate nitrogen recorded as nitrogen in parts per million for the different depths of soil taken in the spring after irrigation and chemical treatment were applied and in August after the wheat had been harvested on the variously treated plots for the 3-year period 1933 to 1935, inclusive*

Treatment	Nitride nitrogen at soil depths indicated (in inches)				
	0–6	6–12	12–24	24–36 [1]	0–36 [1]
SPRING					
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
$Ca(NO_3)_2$	9.0	5.0	7.9	12.6	11.2
$(NH_4)_2SO_4$	4.4	4.3	5.1	9.9	8.3
$(NH_4)H_2PO_4$	4.2	3.8	4.4	9.2	7.3
$MnSO_4$	3.2	2.9	4.9	10.3	8.0
$FeSO_4$	2.7	3.6	5.0	9.5	7.1
No treatment	2.6	2.8	5.2	11.3	8.0
$CaH_4(PO_4)_2$	2.5	2.9	6.1	11.2	8.4
Level of significance	[2] 1.3	1.7	1.7	2.8	1.9
AUGUST					
$Ca(NO_3)_2$	3.0	1.6	1.9	4.9	2.7
$(NH_4)_2SO_4$	2.9	1.5	2.6	3.5	2.4
$(NH_4)H_2PO_4$	3.1	1.8	1.7	3.2	2.4
$MnSO_4$	2.8	1.6	1.9	3.0	2.0
$FeSO_4$	2.9	1.6	2.0	2.9	2.0
No treatment	3.0	1.5	1.8	1.9	1.7
$CaH_4(PO_4)_2$	3.0	1.4	1.9	2.4	1.9
Level of significance	.55	.53	.54	2.1	.8

[1] 2-year average 1933 and 1935.

[2] Each depth was set up as a separate experiment and the standard error obtained by the analysis of variance. A test for homogeneity was applied to a complex experimental set-up for all of the data for nitrogen in the 4 depths of soil. The test in this case is designed to determine whether the observed variances can be considered as having been drawn from the same population. 3 of the depths showed homogeneity but the fourth depth (24–36 inches) indicated lack of homogeneity. All of the data were then analyzed by separate depths. The writers are indebted to Dr. F. R. Immer of the Minnesota Agricultural Experiment Station for the method of testing the data and to Dr. A. E. Brandt of the statistical laboratory, Iowa State College, for developing the method and permitting its use.

The nitrate nitrogen content of the first and second 6 inches is important since the roots of the young seedlings evidently have not penetrated below this depth at this early stage of growth. Weaver (*8, pp. 133–134*) described a month-old Marquis wheat plant as follows:

> On May 1, a month after planting and when the second leaf was half grown, a typical root system was drawn. The number of roots varied from three to eight. Lateral roots were fairly abundant but entirely unbranched. The greatest lateral spread was 5 inches and the working depth or working level (*i. e.* a depth to which many roots penetrate and to which depth considerable absorption must take place) 6.5 inches.

The nitrate nitrogen content of the soil in the calcium nitrate plot was significantly higher than that of any of the other plots. The two sets of plots receiving nitrogen as ammonia were significantly higher than the no-treatment plot, but were not significantly higher than the plots receiving manganese sulphate. In the second 6 inches the amount of nitrate nitrogen in the calcium nitrate plots, while still significantly higher than that of the other plots, was lower than that in the first 6 inches. While the ammonium sulphate plot was some-

what higher than the no-treatment plot, it did not differ significantly from the other treatments. The nitrate nitrogen content of the soil in the second foot was, in all cases, higher than that of the second 6 inches. The calcium nitrate plot was significantly higher than the other plots. In the third foot a noticeable increase of nitrogen is apparent. Tests made in 1934 before and after irrigation indicated that the nitrates were washed below the third foot. The first 6 inches; second 6 inches, and second foot were almost depleted as far as the nitrate tests showed. A slight loss was indicated in the third foot.

No significant differences were noted in the first 6 inches or in the second 6 inches of soil taken from the different plots in August (table 5). In the second foot, however, the plot receiving ammonium sulplate differed significantly from the other plots in parts per million of nitrogen. In the 0–36-inch column a significant difference between the no-treatment and the calcium nitrate plots was found. None of the other plots showed a significant difference.

The yield data indicate that the nitrate added as calcium nitrate increased the yield over the other treatments. The soil-analysis data show that the nitrate nitrogen in the calcium nitrate plots was considerably higher in the first 6 inches. Evidently, a lack of nitrogen at this stage affects the normal development of the young seedlings. The calcium nitrate plots were a much darker green than the other plots.

The plots receiving nitrogen as ammonium acid phosphate outyielded the plots receiving no nitrogen. The nitrate content of the first 6 inches of soil, however, was not significantly different from that of some of the plots receiving no nitrogen. A similar condition was found in the second 6 inches.

The ammonium sulphate-treated plots which differed significantly in yield from the plots receiving no nitrogen showed the same condition in the nitrate content of the soil. Since the nitrate was more effective in correcting the chlorotic condition, it would appeor that nitrogen is more readily absorbed by wheat plants as the NO_3 ion than as the NH_4 ion. The benefits received from the ammonium salts could have been due either to nitrification of these salts or to the direct absorption of ammonia. Nitrification could have been appreciable and still not have been detected in the analyses.

Nitrogen Content of Grain and Straw

The nitrogen content of the grain and straw was determined by the Gunning method (*1*).

The average percentage of nitrogen in the grain is given in table 6.

The nitrogen content of the threshed grain varied slightly for the different treatments. The calcium nitrate plots gave the lowest content in every case, except in 1935. The average content for the 3-year period indicates a significant difference over the no-treatment plots for the plots receiving manganese sulphate and ammonium phosphate.

The nitrogen in the straw was considerably lower than in the grain (table 6).

The nitrogen in the straw was low in the ammonium-treated plots. No significant difference was noticed between the other treatments. All of the other plots were significantly higher than the ammonium acid phosphate and ammonium sulphate plots.

TABLE 6.—*Average yearly nitrogen content of grain and of straw from the differently treated plots for 1933 to 1935, inclusive*

GRAIN

Treatment	1933	1934	1935	3-year average
	Percent	*Percent*	*Percent*	*Percent*
$MnSO_4$	2.80	2.74	2.58	2.71
$(NH_4)H_2PO_4$	2.79	2.76	2.58	2.71
$FeSO_4$	2.79	2.74	2.58	2.70
$(NH_4)_2SO_4$	2.79	2.73	2.54	2.68
$CaH_4(PO_4)_2$	2.76	2.73	2.54	2.68
No treatment	2.76	2.73	2.52	2.67
$Ca(NO_3)_2$	2.63	2.73	2.61	2.66
Level of significance	.071	.056	.066	.037

STRAW

No treatment	0.49	0.43	0.52	0.48
$Ca(NO_3)_2$	.45	.46	.56	.48
$CaH_4(PO_4)_2$	.48	.41	.56	.48
$MnSO_4$	.48	.41	.51	.47
$FeSO_4$	.47	.40	.51	.47
$(NH_4)H_2PO_4$	.47	.42	.47	.45
$(NH_4)_2SO_4$	.45	.43	.46	.44
Level of significance	.028	.035	.035	.019

EFFECT OF TEMPERATURE OF IRRIGATION WATER AND FERTILIZER TREATMENTS ON THE DEVELOPMENT OF CHLOROSIS

As previously stated, the mild chlorotic condition often found in small grains after heavy rains or applications of irrigation water to the plants in the earlier stages of growth has been attributed to various causes, one of the commonest being the temperature of the irrigation water. From the tests described above, there is no indication that water at a temperature as low as 40° F. has any more detrimental effect on the plants than water at 60°. The temperature of the soil was affected more by the temperature of the surrounding air than by the temperature of the irrigation water between the range of 40° to 60°.

An application of calcium nitrate immediately after irrigation prevented the occurrence of chlorosis. A similar but less pronounced effect was obtained when ammonium sulphate or ammonium acid phosphate was applied. No difference in the color or type of growth was noticed in the plants receiving manganese sulphate, iron sulphate, and superphosphate. These results indicate that the chlorotic condition resulted from a shortage of nitrogen rather than from any other elemental deficiency. Nitrate proved more effective than ammonium salts in controlling the chlorotic condition, indicating a greater availability of the nitrate ion.

When the nitrate nitrogen content of the soil in the first 6 inches was low, chlorosis resulted. When the content was high, as in the plot treated with calcium nitrate, no chlorosis occurred. Ammonium nitrogen had a similar effect, but to a less degree.

SUMMARY

Tests conducted with Marquis wheat irrigated at germination with 6 inches of water have shown that:

(1) The temperature of irrigation water ranging from 40° to 60° F. has no effect on the yield of grain or straw.

(2) The addition of nitrogen fertilizer to the crop immediately after irrigation prevents the chlorotic condition often observed in young wheat plants after heavy rains or the application of irrigation water and increases the yield of grain.

(3) The irrigation water washes the soluble nitrate nitrogen below the 3-foot level.

(4) Yields both of straw and grain are increased by applications of calcium nitrate, ammonium acid phosphate, and ammonium sulphate in the order named.

(5) In plots treated with calcium nitrate, the nitrate nitrogen content was high in the first 6 inches of soil. In plots receiving no nitrogen the nitrate nitrogen content was significantly lower.

(6) Applications of manganese sulphate, iron sulphate, and superphosphate did not alter the chlorotic condition or increase the yield over the no-treatment plots.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
[1931]. official and tentative methods of analysis . . . compiled by the committee on editing methods of analysis. Ed. 3, 1930. 593 pp., illus. Washington, D. C.

(2) Fisher, R. A.
1932. statistical methods for research workers. Ed. 4, rev. and enl., 317 pp., illus. Edinburgh and London.

(3) Gardner, R., and Robertson, D. W.
1935. the use of sugar beet petioles as indicators of soil fertility needs. Colo. Expt. Sta. Tech. Bull. 14, 16 pp.

(4) Patten, H. E.
1909. heat transference in soils. U. S. Bur. Soils Bull. 59, 54 pp., illus.

(5) Roberston, D. W., Kezer, A., Sjogren, J., and Koonce, D.
1934. studies on the critical period for applying irrigation water to wheat. Colo. Expt. Sta. Tech. Bull. 11, 43 pp., illus.

(6) Snedecor, G. W.
1934. calculation and interpretation of analysis of variance and covariance. 96 pp. Ames, Iowa. (Iowa State Col., Div. Indus. Sci. Monog. 1.)

(7) Sweet, A. T., and Spencer, J. N.
1931. soil survey of the fort collins area, colorado. U. S. Bur. Chem. and Soils, Soil Survey Rept. Ser. 1927, no. 27, 29 pp., illus.

(8) Weaver, J. E.
1926. root development of field crops. 291 pp., illus. New York.

FACTORS INFLUENCING THE RIPENING SEASON OF SOUR CHERRIES [1]

By V. R. GARDNER

Director, Michigan Agricultural Experiment Station

INTRODUCTION

With cherries, as with most other fruits, the season at which the fruit matures is important in determining its market value. Thus, varieties such as Black Tartarian, which would be of little worth if their fruit were to ripen 2 weeks later than it does, are highly prized because they are among the first to reach the market.

In a general way each variety has its characteristic ripening season, and efforts to advance, retard, shorten, or lengthen the harvesting season have been limited largely to the selection of varieties that would appear to meet the requirements in question. Nevertheless, there is considerable variation in ripening season within the same variety, not only from place to place but even within the same orchard. Thus in the Graham station orchard near Grand Rapids, Mich., which furnished many of the data for this report, the first picking of Montmorency in 1935 was made on July 16 and the last on September 14. Sometimes this prolonged ripening season occasions no great inconvenience or loss, but at other times it may entail considerable added expense because of the necessity of fractional picking or, if such picking is not employed, it may cause considerable loss in revenue because of the change in price as the season advances, or the lower price received for a product not uniform in degree of maturity.

For commercial canning only cherries that are fully tree-ripened are wanted. Those that are slightly immature lose too much of their color in processing and the appearance of the finished product is impaired. If the fruit is left on the trees until all of it is ripe enough for canning, there is loss from overmaturity and decay, attack by birds, bruising from the wind, wilting, and other causes. There is perhaps a wider range in the degree of maturity that is acceptable for the fresh-fruit trade, but uniformity within the package is highly desirable. Experience shows that in many seasons in certain Montmorency orchards fully 3 weeks intervenes between the first and the last pickings when an effort is made to meet the demands of an exacting trade.

It has seemed desirable, therefore, to make a study of some of the more important factors that influence the season of ripening of cherries and to discover, if possible, practicable means of advancing or retarding and of making more uniform the season of maturity.

REVIEW OF LITERATURE

Though there is a fairly extensive literature pertaining to cherries and cherry culture, data relating to factors other than variety that influence the ripening season are rather limited.

[1] Received for publication Feb. 4, 1937; issued October 1937. Journal article no. 289 (n. s.) from the Michigan Agricultural Experiment Station.

Growers generally are aware of the influence of latitude on the ripening season of fruits. Thus in Michigan the harvesting of Montmorency usually takes place during the first half of July in the more southern counties, during the second and third weeks in the central counties, during the last half of the month in the Traverse City area, and during early August in the northernmost sections that are subject to the influence of Lake Michigan. This difference is considerably greater than the average of 3.6 days retardation in ripening season for each degree of latitude found by Phillips (*9*) [2] as characterizing the Montmorency cherry in the Mississippi Valley. That this influence of latitude on ripening season is probably in large part a temperature effect is indicated not only by the prevailing temperatures at these different latitudes, but by the experience of English and other northwestern European growers who have been able to advance the ripening season of some cherry varieties 2 or 3 weeks by training them as espaliers or cordons along the south side of walls (*11*) and to retard ripening for a similar period by shading (*2*).

With many fruits soil exerts a very pronounced influence on ripening season, light soils tending to promote maturity and heavier soils tending to retard it. This influence is not so marked in the case of cherries, though it has been noted many times (*13*). The application of nitrogenous fertilizers tends to delay maturity in many fruits. This influence has been observed with sour cherries (*12*), but there is some question as to its relative importance. Fertilization has also been noted (*7*) as contributing to the evenness of ripening of cherries. Though rootstocks undoubtedly exert some influence on earliness and evenness of ripening, (*5*), data are not available to indicate clearly what stocks may be used with confidence to advance or to retard ripening.

Little attention has been given to the importance of intravariety strains in the case of cherries, though there are a number of references in the literature to the occurrence of limb sports that differ from the parent tree in ripening season. May Duke in particular has long been noted as a variety characterized by very uneven ripening (*1*, *3*), apparently due to what might be called an ever-sporting tendency, and Hochgenuss von Erfurt (*10*) is apparently another variety of the same type. A number of whole tree and limb variations in season of ripening in the Montmorency variety have been described recently by Drain (*4*), and the presumption is that directly or indirectly other variations of this same type have been responsible for more or less unevenness in ripening in the general run of stock propagated commercially by the nurseries.

MATERIALS AND METHODS

There were available for the purposes of this study the 1931–34 harvesting records of each of 194 cherry trees (*Prunus cerasus* L.) of the Montmorency variety in the so-called Corporation orchard near South Haven, Mich., and the 1931–36 harvesting records of each of 149 trees in the Graham station orchard near Grand Rapids, both of which orchards had been set in the spring of 1920. The records for the Corporation orchard included individual tree yields each year and a series of notes made during the ripening season in which each tree

[2] Reference is made by number (italic) to Literature Cited, p. 532.

was classified as ripening its fruit evenly or unevenly and relatively early, midseason, or late in the season. In the Graham station orchard fractional picking was employed each year on those trees that ripened unevenly and records were kept of the quantities harvested at each picking. Special effort was made each year to harvest the fruit at as nearly the ideal stage of maturity as possible. Measurements were made of the trunk circumference of each tree in the two orchards each autumn, and the crops of individual trees were classified as small, medium, large, or very large if they totaled as follows for trees of the following trunk circumferences (as measured in the fall of 1933):

Trunk circumference (inches):	*Yield (pounds) and crop classification*
Less than 19	Less than 25, small.
Do	25–75, medium.
Do	More than 75, large.
Do	More than 150, very large.
19–23	Less than 75, small.
19–23	75–100, medium.
19–23	125–200, large.
19–23	More than 200, very large.

This is, of course, an arbitrary classification, but it permits a grouping that brings out the influence of relative size of crop on season of maturity.

In addition to the 149 trees at the Graham station already referred to there were a number of others in other blocks, some younger and some older, which were submitted to various experimental treatments and on which records relating to season of maturity were obtained.

One series of records was obtained in 1933 from four representative individuals in a block of fully mature Early Richmond trees in the orchard of the W. R. Roach Co., of Hart, Mich. These trees were large for their age, healthy, and at an earlier stage in their development had been very vigorous. For several years before the records were taken they had been bearing very heavy crops, so heavy in fact that a considerable percentage of the crop had not been sizing up and ripening properly. Fractional picking had been employed each year, but part of the crop never reached a degree of maturity that made it acceptable for commercial canning, even though allowed to remain on the trees until after the Montmorency harvest had been completed.

Temperature data to compare with the fruiting records of the Graham station trees were obtained from the Grand Rapids office of the United States Weather Bureau, located about 4 miles from the orchard.

INFLUENCE OF TEMPERATURE AND SIZE OF CROP ON RIPENING SEASON

Perhaps the most obvious variation in ripening period encountered in this study was that associated with season. In 1931, 1935, and 1936 cherries ripened late, in 1932 relatively early, and in the other years about midseason. The ripening season in 1934 was very uniform; in 1935 and 1936, uneven and long drawn out, and in the other years intermediate. These facts are well brought out in figure 1 and table 1 for the trees in the Graham station block.

The seasonal variations in earliness or lateness of ripening can be explained in part by the accompanying temperatures. The three relatively early seasons, 1932–34, were characterized by at least moderately high mean May, June, and July temperatures and by corre-

spondingly high heat accumulations (measured by daily maxima) during these months. Though the mean July temperatures and July heat accumulations of the 3 late-maturing years were as high as or higher than those of the 3 early-maturing years, May temperatures and heat accumulations were markedly lower in 1931 and 1935 and June temperatures were markedly lower in 1935 and 1936. The fruit, therefore, had advanced considerably further in its development by July 1 in some years than in others.

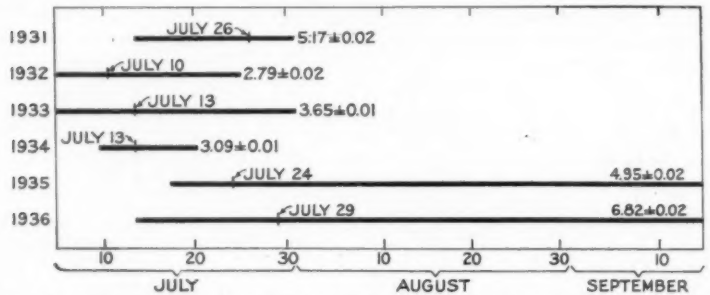

FIGURE 1.—Ripening season of fruit on 149 trees at the Graham station, Grand Rapids, Mich., 1931–36. Vertical lines indicate mean picking dates; figures at right end of horizontal lines indicate standard deviations, in days, in harvesting dates.

TABLE 1.—*Yields and ripening dates of 149 Montmorency cherry trees at the Graham station, 1931–36, and temperature records for the growing seasons of those years*

Year	Average yield per tree	First picking	Last picking	Average harvest date	Standard deviation in ripening date	Mean temperature			Heat accumulation [1]		
						May	June	July	May	June	July
	Pounds				*Days*	°*F.*	°*F.*	°*F.*	°*F.*	°*F.*	°*F.*
1931	130.6	July 14	July 31	July 26	5.17±0.02	56.0	71.3	75.8	744	1,188	1,379
1932	44.7	July 5	July 23	July 10	2.79±0.02	59.4	70.5	73.0	855	1,170	1,280
1933	109.4	...do.....	Aug. 1	July 13	3.65±0.01	59.7	74.0	75.6	833	1,290	1,373
1934	155.8	July 9	July 19	...do.....	3.09±0.01	63.7	73.3	76.8	1,050	1,287	1,450
1935	100.2	July 17	Sept. 14	July 24	4.95±0.02	53.3	64.8	76.5	644	948	1,360
1936	242.7	July 14	...do.....	July 29	6.82±0.02	62.9	66.2	77.3	1,001	1,101	1,472

[1] Heat accumulation figured as the total of the day degrees of maximum temperature exceeding 41° F.

Examination of the data presented in table 1, however, suggests that size of crop may have had some influence on season of maturity. Thus in 1936, when very heavy crops were borne, the maturing season was very late; and in 1931, another year of fairly heavy crops, the ripening season was again late. The years 1932 and 1933 were years of light or moderate production and the ripening seasons were comparatively early. On the other hand, in 1934 the trees bore heavier crops than were borne the two preceding seasons and they matured their fruit as late or slightly later even though temperatures were higher, while in 1935 with only a medium crop they matured their fruit late. The influence of size of crop upon season of ripening is more clearly indicated by the data in table 2, in which the harvesting records for several seasons of the individual trees in the Corporation and Graham station orchards are classified as to size, earliness, and evenness of ripening.

It will be noted that in both orchards there was a marked tendency for trees bearing relatively light crops to mature their fruit early and for the fruit to ripen evenly, whereas the trees bearing relatively heavy crops matured their fruit late and the fruit ripened more or less unevenly. Trees with medium-sized crops were intermediate in respect to both time and uniformity of ripening. This influence of size of crop is given a quantitative expression by the data presented in table 3. Presumably the retarding effect of a heavy crop was more important in causing late ripening in the Graham station orchard in 1936 than the somewhat subnormal mean temperature during June, for temperatures during the remainder of the growing season were up to or above the average.

TABLE 2.—*Classification of the individual tree yields on a basis of ripening season, crop yield, and manner of ripening, in Corporation and Graham station orchards*

[Each tree each year was classified as bearing a light, medium, heavy, or very heavy crop; as having an early, midseason, or late ripening season; and as having fruit that ripened evenly or unevenly]

CORPORATION ORCHARD, 1931–34 [1]

Kind of crop and manner of ripening	Trees	Percent of total	Kind of crop and time of ripening	Trees	Percent of total
	Number			*Number*	
Light:			Light:		
Even	304	83	Early	115	43
Fairly even	12	3	Midseason	145	55
Uneven	51	14	Late	6	2
Total	367	100	Total	266	100
Medium:			Medium:		
Even	129	45	Early	35	14
Fairly even	84	29	Midseason	188	76
Uneven	73	26	Late	25	10
Total	286	100	Total	248	100
Heavy:			Heavy:		
Even	8	9	Early	0	0
Fairly even	22	26	Midseason	27	56
Uneven	56	65	Late	21	44
Total	86	100	Total	48	100

GRAHAM STATION ORCHARD, 1931–36

Light:			Light:		
Even	129	89	Early	89	61
Fairly even	10	7	Midseason	52	36
Uneven	6	4	Late	4	3
Total	145	100	Total	145	100
Medium:			Medium:		
Even	156	62	Early	115	46
Fairly even	43	17	Midseason	122	48
Uneven	52	21	Late	14	6
Total	251	100	Total	251	100
Large:			Large:		
Even	171	52	Early	55	17
Fairly even	62	19	Midseason	172	53
Uneven	94	29	Late	100	30
Total	327	100	Total	327	100
Very large:			Very large:		
Even	8	5	Early	2	1
Fairly even	61	38	Midseason	83	52
Uneven	92	57	Late	76	47
Total	161	100	Total	161	100

[1] In 1931 the crops in the Corporation orchard were not classified as to early, midseason, or late and again in 1934 only a part was so classified.

TABLE 3.—*Ripening-season data for individuals of selected groups of Montmorency cherry trees in the Graham station orchard, 1931–36*

[The figures presented are the averages for the 25 lowest and 25 highest yielding individuals of each year, regardless of what those individual trees may have yielded in other years. The 50 individuals were the extremes from the standpoint of yield of the 149 trees in the orchard]

Year	The 25 lowest-yielding trees [1]			The 25 highest-yielding trees [1]		
	Average yield	Average ripening date	Averages of standard deviations in ripening date for each of the 25 trees	Average yield	Average ripening date	Averages of standard deviations in ripening date for each of the 25 trees
	Pounds		*Days*	*Pounds*		*Days*
1931	59	July 19	4.80	195	July 28	4.01
1932	7	July 8	2.44	97	July 10	3.76
1933	49	July 11	1.70	175	July 14	2.10
1934	46	July 10	.....	262	July 16	.40
1935	48	July 23	1.25	165	July 25	1.30
1936	125	July 27	4.20	346	July 29	5.30

[1] It should be made clear that each of the 25 trees (say lowest-yielding trees) had a day which was considered to be its "ripening" date (here an average) and that each tree had a "ripening period" standard deviation, or a period in which about two-thirds of its fruit ripened. The averages are merely the averages of these quantities for the 25 trees. Hence the average of the standard deviations is an average of the periods, for each tree, in which about two-thirds of the fruit ripened. This value shows the average period in which about two-thirds of the cherries ripened; it is akin to an average of ripening periods or an average of ranges. This average of the standard deviations is not a standard deviation of the set of items made up of averages of ripening dates.

INFLUENCE OF SHADING AND SIZE OF CROP ON RIPENING SEASON

It has long been a matter of observation that cherries in the tops and on the more exposed outside limbs of the trees often ripen earlier and more evenly than those on the lower and interior limbs. In order to obtain information as to whether this is in the main a size-of-crop or a shading-and-exposure influence several trees were selected shortly before harvest in the summer of 1932 and again in 1933, as follows: (1) Trees with a heavy crop on one side and a light crop on the other, (2) trees with a heavy crop in the top part and a light crop in the bottom, but with no marked difference between the two parts in exposure to light. Fractional pickings were made of the two sides of these trees and of their upper and lower halves, respectively, and great care was taken to harvest fruit only of the same degree of maturity. Still other trees were selected in the same years, all of which were bearing relatively heavy crops, but some of which had (3) dense tops with heavily shaded centers and (4) others which had very open spreading tops with centers well exposed to sunlight. In harvesting the fruit from these last two groups, fractional pickings were made of (*a*) tops and centers and (*b*) inside or center portions. The harvesting records of these four groups of trees are presented in table 4.

TABLE 4.—*The ripening season and yield of Montmorency cherry trees as influenced by the location of the fruit in different parts of the tree, Graham station orchard, 1932–33*

Tree no.	Picking dates	Portion of tree	Yield		Tree no.	Picking dates	Portion of tree	Yield	
			Lb.	*Pct.*				*Lb.*	*Pct.*
15–A	July 8, 1932	East side	39	76	1–C (open top).	July 9, 1932	Outside	36	84
	July 11, 1932	do	12	24		July 12, 1932	do	7	16
	July 8, 1932	West side	14	88		July 8, 1932	Inside	9	50
	July 11, 1932	do	2	12		July 12, 1932	do	8	44
	July 6, 1933	East side	8	11		July 15, 1932	do	1	6
	July 11, 1933	do	48	65		July 7, 1933	Outside	21	19
	July 17, 1933	do	18	24		July 12, 1933	do	51	47
	July 6, 1933	West side	35	69		July 16, 1933	do	37	34
	July 11, 1933	do	16	31		July 12, 1933	Inside	12	50
1–H	July 8, 1932	Top	41	77		July 16, 1933	do	12	50
	July 11, 1932	do	12	23	1–D (dense top).	July 7, 1933	Outside	16	26
	July 8, 1932	Bottom	7	37		July 12, 1933	do	38	61
	July 11, 1932	do	12	63		July 16, 1933	do	8	13
1–B (dense top).	July 9, 1932	Outside	60	75		July 12, 1933	Inside	22	45
	July 12, 1932	do	20	25		July 16, 1933	do	27	55
	July 9, 1932	Inside	2	8	8–H (open top).	July 7, 1933	Outside	17	17
	July 12, 1932	do	20	77		July 12, 1933	do	72	71
	July 15, 1932	do	4	15		July 16, 1933	do	12	12
	July 7, 1933	Outside	9	8		July 12 1933	Inside	30	59
	July 12, 1933	do	72	69		July 16, 1933	do	21	41
	July 16, 1933	do	24	23	9–J (open top).	July 7, 1933	Outside	5	5
	July 12, 1933	Inside	35	58		July 12, 1933	do	60	66
	July 16, 1933	do	25	42		July 16 1933	do	26	29
						July 12, 1933	Inside	18	60
						July 16, 1933	do	12	40

It will be noted that in both years the light-cropping side of tree 15–A matured its fruit somewhat earlier and more evenly than the heavier cropping side. However, both trees (1–H and 15–A) which had been so grown and so pruned that both tops and bottoms were well exposed to light but which bore heavier crops in the tops than in the bottoms, had a tendency to mature their fruit earlier in the tops than in the bottoms in spite of the larger crops. This might lead to the assumption that shading of fruit and foliage in the lower part of the tree was responsible for the later and more uneven ripening. However, the fact that those trees for which "outside" and "inside" picking records were obtained ripened their outside fruit earlier and more evenly than their inside fruit, regardless of whether the tree had a dense or an open top, would indicate that shading within the limits commonly afforded by the tops of cherry trees is perhaps not of so great importance in this connection. Observations as to the distribution and ripening of the fruit in the several parts of these trees, together with the data in table 6, lead unmistakably to the opinion that both earliness and evenness of ripening depended more upon the relative amount of leaf area as compared with the amount of fruit borne by the portions of the tree in question. Where there was a relatively large amount of foliage for the fruit of the limb or part of the tree in question, the ripening season was comparatively early and even; where foliage was limited for the fruit associated with it and to the development and ripening of which it was contributing, ripening was delayed and uneven. This interpretation of ripening behavior ties in with and helps explain some of the seasonal peculiarities in season of maturity mentioned earlier in this paper, e. g., the late and uneven ripening of the 1936 crop, which was extremely heavy.

Even more convincing as to the retarding effect on ripening of a very large crop and of limited leaf surface are the records obtained in 1933 in the Early Richmond block briefly described under Materials and Methods. Four trees considered as fairly representing the conditions of tree growth and crop in this block were selected. At harvest counts were made of the number of cherries on limbs of approximately the same diameter in each of these trees and of the number of leaves accompanying them. The cherries were weighed and classified as to maturity, and leaf areas were determined. Similar records were made for the whole trees. The data obtained are summarized in tables 5 and 6. In general the larger the amount of foliage in relation to the amount of fruit, the more even and complete was the ripening of the fruit. It is realized, of course, that branches of the sizes included in this table are not entirely independent, for there is considerable translocation of food materials from limb to limb in the cherry tree (*8*). The data obtained permit no exact statement as to the number of leaves or the leaf area necessary to mature a cherry, but for Early Richmond in the Roach orchard in 1933 it appears that not less than 5 square inches of leaf area was required for each fruit if it was to ripen properly and that when there was an 8- or 10-square-inch leaf area present per cherry, the chances of those cherries ripening properly at the normal maturing season were good.

TABLE 5.—*Harvesting and leaf records of 4 Early Richmond trees growing near Hart, Mich., arranged in descending order according to apparent vegetative vigor, 1933*

Item	Tree A	Tree B	Tree C	Tree D
Trunk circumference.....inches..	24.5	20.0	20.5	24.0
Leaves, estimated [1].....number..	89,000	68,000	63,000	64,000
Leaf area, estimated [2].....square inches..	293,700	223,000	195,900	202,200
Leaves per fruit, estimated.....number..	3.1	3.2	1.10	1.15
Leaf area, per fruit, estimated.....square inches..	10.30	10.60	6.63	3.66
Total fruits.....number..	28,285	20,976	29,559	55,350
Fruits maturing properly.....do....	18,586	11,149	13,753	16,850
Fruits not maturing properly.....do....	9,699	9,827	15,806	38,500
Total weights of fruit.....pounds..	178.3	138.4	157.6	266.0
Weight of fruit maturing properly.....do....	117.9	72.4	81.1	91.5
Weight of fruit not maturing properly.....do....	60.4	66.0	76.5	174.5
Average cherries per pound.....number..	159	152	188	203
Average cherries per pound maturing properly.....do....	158	154	170	184
Average cherries per pound not maturing properly.....do....	160	150	207	221

[1] Estimate made by counting the leaves on several branches 2 inches in diameter and multiplying that number by the estimated number of branches of equivalent size.

[2] Estimate made by multiplying the estimated number of leaves by the average area of a large random sample.

TABLE 6.—*Size and maturity of fruits from selected branches of Early Richmond trees, as influenced by the associated leaf area, Hart, Mich., 1933*

Tree	Branch no.	Branch diameter	Fruits maturing properly		Average number of fruits per pound maturing properly	Fruits not maturing properly		Average number of fruits per pound not maturing properly	Total leaves	Average number of leaves per fruit	Total leaf area	Average leaf area per fruit	Fruits maturing properly
		In.	*Number*	*Lb.*	*Number*	*Number*	*Lb.*	*Number*	*Number*	*Number*	*Sq. in.*	*Sq. in.*	*Percent*
	1	¾	562	3.3	170	31	0.2	155	1,265	2.1	3,767	6.4	95
	5	¾	320	1.95	164	5	------	--------	700	2.1	2,205	6.8	99
	6	¾	515	3.15	164	69	.4	172	1,808	3.0	5,243	9.0	88
	7	¾	348	2.0	174	123	.75	164	1,126	2.3	3,772	8.0	73
A---	2	¼	91	.55	165	14	.07	200	209	2.0	587	5.6	86
	3	¼	83	.5	166	10	.05	200	336	3.6	1,014	10.9	89
	4	¼	103	.65	159	0	0	--------	182	1.76	551	5.3	100
	8	¼	48	.3	160	21	.1	210	148	2.1	534	7.7	70
	1	¾	84	.5	168	30	.15	200	222	1.9	672	5.9	74
	5	¾	53	.3	177	26	.15	173	234	2.96	720	9.1	67
	7	¾	75	.45	167	45	.25	180	157	1.3	455	3.8	63
B---	3	½	83	.5	166	19	.1	190	219	2.1	650	6.3	81
	2	¾	226	1.4	161	195	1.1	177	454	1.07	1,384	3.3	54
	4	¾	231	1.5	154	30	.2	150	701	2.7	2,046	7.8	89
	6	¾	177	1.2	148	56	.4	140	495	2.1	1,494	6.4	76
	1	¼	70	.4	175	56	.15	373	141	1.1	400	3.2	55
	2	¼	73	.4	182	98	.5	196	214	1.2	588	3.4	43
	4	¼	37	.2	185	71	.25	284	106	.98	269	2.5	34
C---	5	¼	78	.4	195	76	.3	253	202	1.3	559	3.6	51
	3	¾	185	1.1	168	189	.95	199	490	1.3	1,465	3.9	50
	6	¾	334	1.9	176	424	1.9	223	1,012	1.3	2,742	3.6	44
	7	¾	255	1.55	165	217	1.2	181	735	1.5	2,249	4.8	54
	8	¾	407	2.45	166	336	2.0	168	1,418	1.9	4,296	5.8	55
	2	¼	106	.5	212	39	.2	195	259	1.78	880	6.1	73
	3	¼	26	.2	130	114	.6	190	277	1.9	781	5.6	18
	4	¼	39	.15	260	143	.7	204	294	1.1	669	3.7	21
D---	7	¼	108	.55	196	54	.2	270	153	.94	430	2.7	66
	1	¾	201	1.0	201	676	3.1	218	712	.8	2,065	2.4	22
	5	¾	562	2.8	200	314	1.5	210	816	.93	2,407	2.7	64
	6	¾	238	1.2	198	492	2.15	229	509	.7	1,598	2.2	32
	8	¾	397	1.95	204	256	1.1	233	747	1.1	2,181	3.3	60

[1] Correlation coefficient between average leaf area in square inches per fruit and percentage of fruit maturing properly equals 0.64±0.04.

EFFECT OF NITROGENOUS FERTILIZERS ON RIPENING SEASON

The statement is often made that applications of nitrogen-carrying fertilizers have a tendency to delay the ripening of cherries, and many fruit growers are of that opinion. All of the trees furnishing records for this study received annually a moderate application of sulphate of ammonia—from 2 to 4 pounds per tree—the quantity varying with the season and size of tree. The records of these trees therefore did not furnish an answer to the question as to the influence of nitrogen-carrying fertilizers on season of maturity of the fruit. Inasmuch as an earlier study (*6*) of cultural practices in the Montmorency cherry orchard, which included some fertilizer trials, had failed to show any marked influence of applications of nitrogenous fertilizers on ripening season, it was decided to put the idea to a crucial test. Several trees on the Graham station grounds, not included in the block for which ripening-season records have been given, several years older but more or less comparable to them, were treated with heavy applications of sulphate of ammonia each year for 3 successive years. Eighteen pounds per tree per year were applied to some and 25 pounds per tree

to others. These applications were so heavy that they destroyed all vegetation beneath the trees, but apparently they did not injure the tree roots, at least not seriously, for the trees made a very vigorous vegetative growth, blossomed freely, and set and matured heavy crops. Each year the fruit ripened midseason or earlier for Montmorency in that part of the State and the ripening season was remarkably even. The heavy fertilizer applications had no retarding effect on maturity. There was ample foliage throughout the trees for the even and reasonably early maturity of all the fruit that they produced.

In this connection it should be mentioned that the thin, weak wood had been removed from the centers of these trees so that they contained few or no fruiting branches that had set a considerable number of fruits but that did not possess a correspondingly large amount of foliage. Observation leads to the opinion that the idea that the application of nitrogenous fertilizer to cherry trees causes delayed maturity and uneven ripening is due in large measure to the fact that because of the crowding and shading effect of the vigorous branches in the outside and top of the tree, the interior branches become weak, produce little foliage, and then set more fruit than they can mature properly. The result is delayed and uneven ripening on the part of this fruit in the center of the tree. Removal of the slender, weak fruiting wood from the centers would do away with the difficulty.

INFLUENCE OF STRAIN ON EARLINESS AND EVENNESS OF RIPENING

Reference has been made to the occurrence of late-maturing limb or whole-tree sports in cherry orchards. The Corporation orchard, which furnished part of the data for this study, contained one such late-ripening tree. In no year could it be picked when the other trees were being harvested, its harvesting season being about 10 days later. This particular tree has been propagated and some of its progeny brought to bearing age. The daughter trees faithfully reproduce the late-maturing characteristics of their parent, thus furnishing evidence that the late ripening of the parent tree is not due to stock or other environmental influences, but is due to a strain difference. The Graham station orchard likewise contains one late-maturing whole-tree sport, averaging about a week later than the other trees in the same block, and several late-maturing limb sports. The author has noted many such late-maturing whole-tree and limb sports in the cherry orchards of Michigan. They are of frequent occurrence in Montmorency, very frequent occurrence in English Morello, of less frequent occurrence in Early Richmond. A large number of these season-of-maturity sports have been propagated at the Graham station, and they have been found to transmit their characteristics to their vegetative progeny. There can be no question, therefore, as to the existence of late-maturing and early-maturing strains of cherries in the stocks of the three leading sour cherry varieties as they are obtainable from the trade, and there can be no question as to how they have originated. Close observation in hundreds of cherry orchards in Michigan warrants the statement that, though these striking sports are of rather frequent occurrence, they are recognized by the grower for what they are, they are not harvested along with the main crop, in many cases they are not harvested at all, and perhaps on the whole they can be ignored.

The occurrence of these striking season-of-maturity sports, however, raises a question as to whether there are not an equal or greater number of less prominent strains that are not so readily recognized but that, commercially, are more important because they are constantly contributing to lateness or unevenness of maturity. Obviously the very limited number of trees for which harvesting-season records were available for this study are inadequate for a satisfactory answer to this question. It may be noted, however, that, except for the two late-maturing whole-tree sports just mentioned, in no single instance did a tree that ripened its fruit relatively early or relatively late in some one season similarly ripen its fruit relatively early or relatively late in all of the other seasons. Thus, for instance, of the 17 trees out of the entire 149 in the Graham station orchard that matured their fruit earliest in 1931, only 4 matured their crop relatively early in 1932, only 2 in 1933, 7 in 1934, 2 in 1935, and 2 in 1936; furthermore, 11 of the 17 matured their fruit relatively late in 1932, 1 matured its fruit late in 1933, 2 in 1934, 4 in 1935, and 2 in 1936. The influence of the ratio between amount of foliage and size of crop, already discussed, apparently was sufficient to mask any minor strain influences in respect to ripening season.

DISCUSSION

The interpretation to be placed on the data that have been presented is that, though the ripening season of cherries depends in large measure upon growing-season temperatures, both earliness and evenness of ripening are considerably influenced by size of crop and the relative amount of leaf surface that accompanies it. Ripening can be hastened and rendered more uniform by employing such cultural measures as promote a vigorous growth and keep the leaves functioning actively. Pruning, especially the kind that removes weak, slender, interior limbs that are rather poorly supplied with leaves, and the liberal use of nitrogenous fertilizer tend to provide such proper balance between vegetative growth and size of crop, and spraying with such materials as protect the leaves from fungous attack without causing an undue amount of spray injury tends to maintain it.

SUMMARY

The ripening season of cherries in contiguous areas often varies greatly, there being as much as 3 to 4 weeks' difference for the same variety between places 25 to 200 miles apart in the western Michigan fruit belt.

The ripening season of the same variety in the same orchard may vary as much as 2 to 3 weeks from year to year.

Growing-season temperature is a factor of major importance in determining the ripening season of cherries, ripening being delayed by relatively low temperatures.

Variation in ripening season within the same orchard in any one season and likewise from year to year is due in large measure to size of crop and the relative amount of foliage associated with that crop, large crops and limited foliage tending to delay maturity and cause uneven ripening, and, conversely, small crops and abundant foliage tending to promote earliness and cause even ripening.

Cultural measures that tend to establish and maintain a proper balance between crop and vegetative growth tend toward earlier and more even maturing. Such measures include the use of nitrogenous fertilizers, spraying to protect the leaves, and the removal by pruning of small, interior limbs that have a relatively small amount of leaf surface in comparison to their crop.

Contrary to general opinion, the liberal use of nitrogen-carrying fertilizers was not found to lead to delayed maturity and uneven ripening. It has the opposite effect, if proper pruning methods are employed.

Bud sports giving rise to late- or early-maturing strains are of rather frequent occurrence in the sour cherry, but they are of secondary importance to the factors already mentioned in causing delayed or uneven ripening in the crop as a whole.

LITERATURE CITED

(1) ANONYMOUS.
1855. EFFECT OF SPRING FROST ON CHERRY TREES. Gard. Chron. 1855: 454.
(2) ———
1863. RETARDING CHERRIES AN ANCIENT PRACTICE. Gard. Chron. 1863: 1184.
(3) CLITHEROE, T. G.
1851. CHERRIES. *In* Home Correspondence. Gard. Chron. 1851: 821.
(4) DRAIN, B. D.
1932. FIELD STUDIES OF BUD SPORTS IN MICHIGAN TREE FRUITS. Mich. Agr. Expt. Sta. Tech. Bull. 130, 48 pp., illus.
(5) EAST MALLING RESEARCH STATION.
1928. POMOLOGY. East Malling Research Sta. Ann. Rept. (1927) 15 (1): 37–42.
(6) GARDNER, V. R.
1930. MAINTAINING THE PRODUCTIVITY OF CHERRY TREES. Mich. Agr. Expt. Sta. Spec. Bull. 195, 27 pp., illus.
(7) ILLINOIS AGRICULTURAL EXPERIMENT STATION.
1929. PRUNING AND FERTILIZING AFFECT SOUR CHERRIES LITTLE. Ill. Agr. Expt. Sta. Ann. Rept. 42: 224–225.
(8) OVERHOLSER, E. L., and CLAYPOOL, L. L.
1934. THE RELATION OF LEAF AREA PER CHERRY TO PHYSICAL PROPERTIES AND CHEMICAL COMPOSITION. Gartenbauwissenschaft 9 (2): 95–99.
(9) PHILLIPS, H. A.
1922. EFFECT OF CLIMATIC CONDITIONS ON THE BLOOMING AND RIPENING DATES OF FRUIT TREES, N. Y. (Cornell) Agr. Expt. Sta. Mem. 59, pp. 1379–1416, illus.
(10) SCHMIDT, G. C.
1891. CHERRIES. Letter to Gardener's Chronicle. Gard. Chron. (3) 10: 565.
(11) THOMPSON, R.
1835. A REPORT UPON THE PRINCIPAL VARIETIES OF THE CHERRY CULTIVATED IN THE GARDEN OF THE SOCIETY. Roy. Hort. Soc. London Trans. (2) (1831–35) 1: 248–294.
(12) TUKEY, H. B.
1927. RESPONSES OF THE SOUR CHERRY TO FERTILIZERS AND TO PRUNING IN THE HUDSON RIVER VALLEY. N. Y. State Agr. Expt. Sta. Bull. 541, 26 pp., illus.
(13) WYTHES, G.
1896. EARLY AND LATE VARIETIES OF CHERRIES. Gard. Chron. (3) 20: 159–160.

OBSERVATIONS ON THE VARIATIONS IN CYANOGENETIC POWER OF WHITE CLOVER PLANTS [1]

By CHARLES F. ROGERS, *assistant agricultural biochemist*, and O. C. FRYKOLM, *Emergency Relief Administration research assistant, Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station*

INTRODUCTION

Mirande [2] appears to have been the first to note the cyanogenetic property of some individuals of the common white clover, *Trifolium repens* L., which is abundant in lawns and pastures. He barely anticipated Armstrong, Armstrong, and Horton [3] in the announcement of the observation. The last-named authors were seeking to explain the notable differences in nutrient qualities of some adjacent pastures. Because the botanical composition of the pastures was not alone sufficient to explain the observed differences they sought qualitative differences in plants as well as possible peculiarities of the soil to explain the discrepancies. They stated that a kind of "wild white clover" was coming into favor early in the century because of its greater longevity and value in pastures, and that many such plants were cyanophoric, whereas cyanophoric seedlings of the common white clover were rare. Their observations showed that white clover plants from 15 sources varied both in cyanogenetic power and in ability to hydrolyze cyanogenetic glucosides when such glucosides were added to aqueous extracts of the clovers. Only 2 of the 15 groups tested were found to act selectively on different cyanophoric glucosides, the remainder acted either on all or on none of the glucosides.

More recently Askew [4], Doak [5], and Rigg et al. [6] have studied the cyanophoric properties of white clovers in New Zealand. Like Armstrong and his associates, they found that the wild white clovers were cyanophoric, but that the clovers' cyanophoric powers varied widely with location and type. Askew found a seasonal variation in the hydrocyanic acid (HCN) content of white clover, with a tendency for the acid to increase in late summer. Doak [5] concluded that:

> There is considerable correlation between HCN content and type of white clover.
>
> The most highly producing and persistent lines are invariably associated with high HCN content while the poorer short-lived types are low in this respect.

Doak stated also that plants from the same line vary in HCN content. Rigg, Askew, and Kidson [6] found a variation of from 0.0016 to 0.0124 percent of HCN in white clover from 11 sources.

[1] Received for publication Apr. 16, 1936; issued October 1937. Journal Series Paper No. 1423, Minnesota Agricultural Experiment Station. The investigation reported here was made possible by workers supplied on W. P. A. project 1985, Minnesota Works Progress Administration; sponsor, University of Minnesota.

[2] MIRANDE, M. SUR LA PRÉSENCE DE L'ACIDE CYANHYDRIQUE DANS LE TRÈFLE RAMPANT (TRIFOLIUM REPENS L.) Compt. Rend. Acad. Sci. [Paris] 155: 651–653. 1912.

[3] ARMSTRONG, H. E., ARMSTRONG, E. F., and HORTON, E. HERBAGE STUDIES. II.—VARIATION IN LOTUS CORNICULATUS AND TRIFOLIUM REPENS (CYANOPHORIC PLANTS). Roy. Soc. [London], Proc., B 86: 262–269. 1913.

[4] ASKEW, H. O. DETERMINATION OF HYDROCYANIC ACID IN WHITE CLOVER. New Zeal. Jour. Sci. and Technol. 15: 227–233. 1933.

[5] DOAK, B. W. A CHEMICAL METHOD FOR THE DETERMINATION OF TYPE IN WHITE CLOVER. New Zeal. Jour. Sci. and Technol. 14: 359–365, illus. 1933. See p. 364.

[6] RIGG, T., ASKEW, H. O., and KIDSON, E. B. OCCURRENCE OF CYANOGENETIC GLUCOSIDES IN NELSON PASTURE PLANTS. New Zeal. Jour. Sci. and Technol. 15: 222–227. 1933.

In 1934, when the New Zealand work first came to the writer's attention, tests of 15 plants of white clover for HCN had proved to be all negative, but potted New Zealand white clover seedlings were consistently cyanogenetic. Early in May 1935, preliminary tests of 30 clover plants growing on the campus of University Farm, St. Paul, Minn., showed 6 to be strongly cyanogenetic. Five hundred plants were permanently labeled by June 24, 1935. This study was continued until early in August when further tests for 1935 had to be abandoned.

TECHNIQUE AND RESULTS

The technique employed in the tests was as follows: Six mature leaves (18 leaflets) of each clover plant were put into a 6- by ¾-inch test tube, frozen at −15° C. Nearly saturated picric acid (0.05 molar) was poured over sodium bicarbonate until all of the solid dissolved. If any precipitate formed within 2 minutes more picric acid was added. A layer 2 mm deep was poured into a Petri dish, and white filter paper in strips 3 mm wide was momentarily dipped straight down into the solution, then laid on glass rods for the solution to soak up the paper and partly dry out.

One milliliter of water was measured from a burette into each tube containing frozen clover leaves. The test strip was inserted into the tube, and held in place by the cork. These were allowed to stand at room temperature (22° to 28° C.) for 1 day, when they were set into water at 50° for 15 minutes to volatilize HCN in the solution. The papers were removed and compared immediately with the standards set for scaling purposes. This is a modification of the Guignard [7] test.

As interest was centered in the groups of cyanophoric plants rather than in the exact amount of hydrocyanic acid in each plant, the test papers which indicated the unquestioned presence of hydrocyanic acid in the clover plants were separated into only five groups (table 1). The records entered in table 1 under "Trace" indicate a color of the paper between that of a negative test (paper the original yellow) and definitely reddened tinge to the yellow or strongly colored paper, which is taken as proof of presence, and a measure of quantity of hydrocyanic acid present.

The conditions of soil, moisture, and light exposure, although not identical within each plot, were much alike.

It is seen from table 1 that the greater portion of the plants in any location and at any time were negative in tests for hydrocyanic acid. About two-thirds of the total number tested were negative. It might be expected that these tests were grouped largely about some plants consistently negative in all tests. Plants consistently negative were grouped together, as were also those consistently positive and those intermediate ones which showed both negative and positive reactions. The number of tests made on each plant is given in table 2, and the plant is entered in the line corresponding to number of tests. Thus, if a plant was tested five times and found to be positive three times and negative twice, it would be entered in the column for positive three times. When only a trace appeared one or more times, the plant would be entered in the column headed "Trace." Such

[7] GUIGNARD, L. LE HARICOT À ACIDE CYANHYDRIQUE, PHASEOLUS LUNATUS L. Compt. Rend. Acad. Sci. [Paris] 142: 545-553. 1906.

plants are essentially negative. All groups of plants were tested at least three times, but in each group some plants were lost by the destruction of stakes, and other plants had to be assigned the same number. This accounts for the 39 plants tested less than three times, and 133 plants tested three times.

TABLE 1.—*Distribution of hydrocyanic acid in clover plants according to location of plants*

Plant group nos.[1]	Number of tests		Plants testing—						
	Total	Per plant	Negative	Positive (classified as to approximate intensity) [2]					
				Trace	1	2	3	4	5
0–99	420	5	278	13	45	46	23	13	2
100–199	388	4	289	23	34	18	20	2	2
200–299	299	3	204	18	36	19	14	8	0
300–399	384	4	217	13	48	57	40	8	1
400–499	492	5	297	21	79	47	28	12	8
Total	1,983		1,285	88	242	187	125	43	13
Percentage of total tests			64.80	4.4	12.20	9.43	6.30	2.17	0.66

[1] Site of plants and soil types: 0–99, highland, various types of soil and moisture, old and young plants; 100–299, flat, shallow, sandy soil over gravel bed, old stand; 300–399, sloping sandy soil, thin sod 7 years old; 400–499, black soil, bottom land of pasture, old sod.

[2] "Trace" indicates a color of the paper in between definite negative and definite proof of the presence of hydrocanic acid in appreciable quantity; classifications 1 to 5 indicate hydrocyanic acid in increasing quantities.

TABLE 2.—*Consistency in the cyanogenetic property of white clover plants as measured by the Guignard test-paper method* [1]

Plants tested (number)	Tests	Plants testing—													
		Negative		Positive (classified as to approximate intensity)											
				Trace		1		2		3		4		5	
	Number	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
149	5	53	35.6	2	1.34	25	16.8	17	11.4	27	18.1	9	6.04	16	10.7
192	4	82	42.7	10	5.21	34	17.7	26	13.5	21	10.9	19	10.00	------	------
133	3	57	42.9	9	6.77	36	27.1	16	12.0	15	11.3	------	------	------	------
27	2	20	74.1	------	------	4	14.8	3	11.1	------	------	------	------	------	------
12	1	9	75.0	------	------	3	25.0	------	------	------	------	------	------	------	------

[1] GUIGNARD, L. See footnote 7.

The percentages of plants giving a negative reaction in the groups tested five, four, and three times agree rather well. This suggests that there there are mixed in the sods at University Farm a larger portion of noncyanogenetic white clovers than cyanogenetic. There is also a group of plants similar in general appearance but which gives consistently positive tests for HCN. Some plants varied in cyanogenetic power, but the preponderance of plants was consistently noncyanogenetic. Although not specifically shown in tables 2 or 3, there was no detectable consistency among those plants with one or two positive tests for HCN, for many were positive at first, but negative in later tests, and some were negative at first but positive in one or two later tests. Individually variable plants showed no consistency in their tests.

TABLE 3.—*Tests of cyanogenetic power of white clover on University Farm campus, on various dates during the summer of 1935*

Date	Plant group nos.	Total tests	Plants testing as indicated						
			Negative	Positive, classified as to approximate intensity					
				Trace	1	2	3	4	5
May 9	0- 99	91	75	3	6	6	1		
May 13	0- 99	81	62	2	6	8	1	2	
May 19	400-499	99	71	10	14	4			
May 21	0- 99	79	39	2	14	13	8	3	
May 22	400-499	100	61	1	13	15	8	2	
May 24	100-199	100	84	4	3	3	4		2
Period total		550	392	22	56	49	22	7	2
Percentage		100.00	71.28	4.00	10.18	8.91	4.00	1.27	.36
June 7	0- 99	85	46	5	13	9	7	3	2
Do	400-499	99	64	8	14	8	3	1	1
June 19	100-199	96	73	8	7	4	3	1	
Do	200-299	100	63	9	13	6	4	5	
June 24	300-399	99	77	2	3	9	8		
June 27	100-199	100	77	5	8	5	4	1	
Do	200-299	99	89	0	2	3	4	1	
July 3	300-399	98	39	3	11	23	18	3	1
Do	400-499	98	43	0	23	13	7	5	7
Period total		874	571	40	94	80	58	20	11
Percentages		100.00	65.33	4.58	10.76	9.15	6.63	2.29	1.26
July 16	0- 99	84	56	1	6	10	6	5	
Do	300-399	97	47	2	18	15	11	4	
July 29	200-299	100	52	9	21	10	6	2	
July 30	100-199	92	55	6	16	6	9		
July 31	400-499	96	58	2	15	7	10	4	
Aug. 1	300-399	90	54	6	16	10	3	1	
Period total		559	322	26	92	58	45	16	0
Percentages		100.00	57.60	4.65	16.46	10.38	8.05	2.86	
Grand total		1,983	1,285	88	242	187	125	43	13
Percentage		100.00	64.80	4.44	12.20	9.43	6.30	2.17	.66

Although this work was stopped about the middle of the normal Minnesota growing season, the dry, hot weather after good spring and early summer rains permitted the observation of the effect of unfavorable growing conditions on the cyanogenetic activity of the clovers. Tests were made from May 9 to August 1, 1935, as shown in table 3, where the dates and number of tests of each group are tabulated against the progress of the season.

From the data in table 3 which lists the tests in calendar order one notes a tendency for the number of negative tests of all groups to decrease. Subtotals are taken to show the possible differences in groupings with the advance of the season, utilizing the greatest intervals between groups of tests and changes in weather. The first group of tests was made during cool, moist spring weather; the second or middle group during hot, humid weather when vegetation was flourishing; and the last during a severe drought.

The percentage of negative tests decreased appreciably with the advance of the season from 71.28 to 57.60. There was some rise in the number of plants mildly cyanogenetic, and a tendency to maintain or increase throughout the cyanogenetic activity in all plants but

those highest in HCN. This observation seems to confirm those of Askew.[8]

If the idea held by many, that cyanogenetic activity is induced or accelerated by conditions unfavorable for gross plant growth then the conditions of July should have induced even greater cyanogenetic activity than is apparent, as the increase over the second period is only slightly greater than the increase of the second over the first. The clover plants in many cases were badly stunted by the time the last test material was taken.

SUMMARY AND CONCLUSIONS

To determine the cyanogenetic property of white clover (*Trifolium repens* L.), from 1 to 5 individual tests were made on 513 different white clover plants. In all, 1,983 separate tests were performed between May 9 and August 1, 1935, of which about two-thirds were negative.

Approximately 40 percent of the plants tested three or more times were consistently negative.

Only 10 percent of the plants tested three or more times were consistently positive, and these were usually strongly positive.

There is an indication of increase in the cyanogenetic power of plants with increase in size of plant, with progress of season, and with a generally decreased moisture supply.

[8] ASKEW, H. O. See footnote 4.

MACROSPOROGENESIS AND EMBRYO-SAC DEVELOPMENT IN EUCHLAENA MEXICANA AND ZEA MAYS[1]

By D. C. COOPER [2]

Assistant professor of genetics, Wisconsin Agricultural Experiment station

INTRODUCTION

Studies of macrosporogenesis and the development of the macrogametophyte in the Maydeae are almost wholly limited to corn (*Zea mays L.*), and little is known concerning these stages in the life history of other members of this tribe. True (*12*),[3] Guignard (*7*), and Poindexter (*9*) described the ovules of corn, observed double fertilization, and briefly outlined the early stages in the development of the embryo. Weatherwax (*13*, *14*, *15*) and Miller (*8*) studied the details of macrosporogenesis and embryo-sac development. Later Weatherwax (*17*) compared the development of the endosperm of *Coix* (*Coix lacryma-jobi*) with that of corn. More recently Randolph (*10*) has presented an account of the developmental morphology of the caryopsis of maize in which embryogeny, endosperm formation and structure of the pericarp are fully described. The detailed cytological studies of chromosome morphology in teosinte (*Euchlaena mexicana* Schrad.) and teosinte-corn hybrids (Beadle (*2*, *3*); Emerson and Beadle (*6*); Arnason (*1*)), as well as the genetical analyses of such hybrids, warrant a comparative study of macrosporogenesis and embryo-sac development in the two parents and the hybrid.

MATERIALS AND METHODS

The annual type of Florida teosinte was used in the present investigation. This variety, at the latitude of Madison, Wis. (43°), does not come into flower under field conditions until late in the fall. In order to force the plants into flower so as to make the desired crosses with corn, the seeds were planted in 8-inch pots in the greenhouse about the middle of April. The pots were taken to the field about May 15, the precise date depending upon the weather, and sunk into the ground so that the tops of the pots were flush with the level of the soil. A short-day treatment such as was described by Emerson (*5*) was begun at this time. This was accomplished by placing barrels over the plants at 5 o'clock each afternoon and removing them at 7 o'clock the next morning. This practice was continued until the plants began to blossom (about July 15), when the barrels were removed entirely. Plants treated in this manner flowered abundantly and set a good crop of seed. Reciprocal hybrids between yellow dent corn and teosinte were obtained.

Pistillate spikelets of teosinte and of corn of various ages were collected during the summer of 1934, dipped in Carnoy's fluid for a

[1] Received for publication Apr. 10, 1937; issued October 1937. Paper from the Department of Botany and the Department of Genetics (no. 211), Wisconsin Agricultural Experiment Station.

[2] The writer desires to express his appreciation for support received from the Wisconsin alumni research fund during the period of these investigations.

[3] Reference is made by number (italic) to Literature Cited, p. 550.

short time (one-half to 1 minute), and transferred directly to Karpechenko's modification of Nawaschin's fluid. The material was allowed to remain in the latter fluid from 36 to 48 hours, then washed, dehydrated, and embedded in 52° paraffin in the usual manner, cedar oil being used as the clearing agent. After embedding, longitudinal sections were cut at thicknesses varying from 12μ to 20μ. The older material was cut thicker in order to obtain the embryo sac in as few sections as possible. The sections were mounted serially, stained in dilute Delafield's haematoxylin, and counterstained in safranine. This combination is valuable because of the clear differentiation of the cytoplasm, spindle, and forming cell plate.

OBSERVATIONS

EUCHLAENA MEXICANA

Development of the Ovule

The single ovule first appears as an erect, rounded protuberance at the base of the carpel, later becoming more or less conical in shape. An apical hypodermal cell becomes differentiated as the primary archesporial cell while the ovule is in an upright position. This cell (fig. 1, *A*, *a*) differs from its neighbors in being conspicuously larger, with a somewhat denser cytoplasm and a larger nucleus (fig. 1, *A*). Shortly after the differentiation of the primary archesporial cell the integuments develop as outgrowths of the epidermis near the base of the ovule on the side away from the central axis of the pistillate spike. The inner integument (*i*) is the first to appear and shortly after its initiation and just below it the primordium of the outer integument starts development. The entire ovule grows more rapidly on the side on which the integuments first appear so that it bends toward the main axis of the spike (fig. 1, *D*). The bending takes place in the region of the origin of the integuments and continues until the mature ovule assumes an amphianatropous position, i. e., it is a type intermediate between the amphitropous and the anatropous forms but approaches the latter (fig. 2, *L*).

The inner integument, which remains two layers of cells in thickness, except at the apex in the mature stages, grows so that it has reached a level even with the tip of the ovule by the time the four spores are formed as a result of macrosporogenesis (fig. 2, *E*). The ovule at this time is so bent that the longitudinal axis of the row of spores makes an angle of approximately 45° to the pedicel. The integument continues to elongate, growing slightly beyond the apex of the nucellus and forming a very short micropyle (fig. 2, *L*). The apical portion in the region of the micropyle becomes four or five layers of cells in thickness by the time the ovule is mature. The outer integument likewise remains two layers of cells in thickness except at the apex, where it may be three or four cells in thickness. It does not develop sufficiently to cover more than two-thirds of the ovule.

The archesporial cell becomes the macrospore mother cell without further division and remains adjacent to the epidermal layer of the ovule until the nucleus shows advancing stages of the meiotic prophases (fig. 1, *B*, C). At later stages the cells of the epidermis divide to form three or four layers of nucellar tissue beyond the spore mother cell (fig. 1, *D*, *E*, *F*).

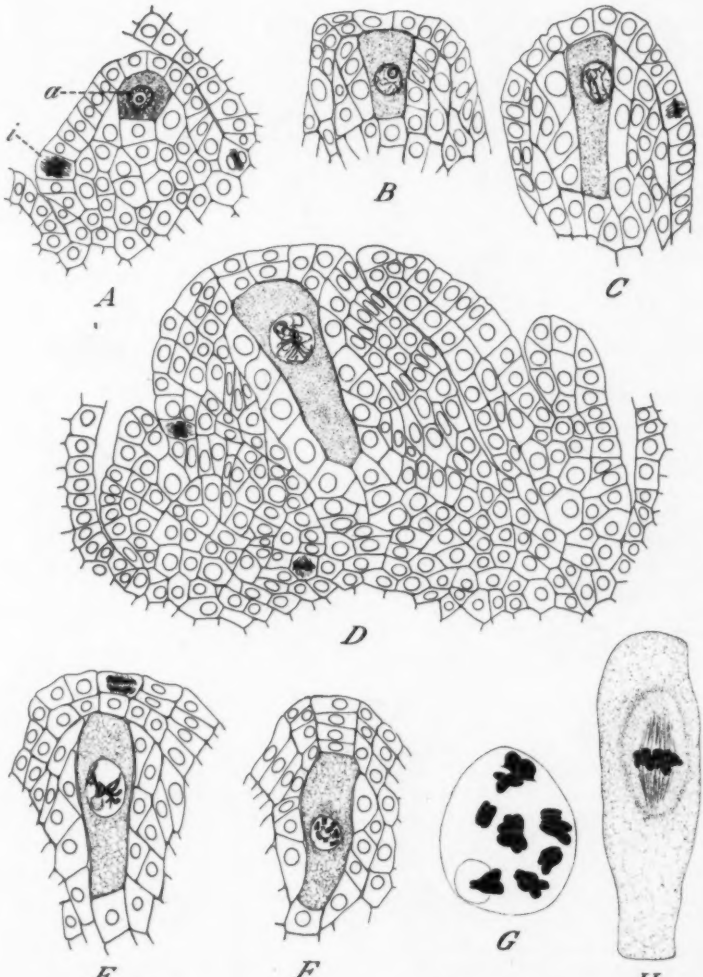

FIGURE 1.—*Euchlaena mexicana: A*, Young ovule; *a*, archesporial cell; *i*, first evidence of integument formation; × 425. *B*, Portion of nucellus with developing archesporial cell; × 425. *C*, Archesporial cell or macrospore mother cell at the onset of meiosis, × 425. *D*, Young ovule with developing integuments. Nucleus of macrospore mother cell at a late spireme stage, × 425. *E*, Portion of nucellus with macrospore mother cell. Nucleus at diplonema, × 425. *F*, Same as *E*, nucleus at diakinesis; × 425. *G*, Nucleus of macrospore mother cell, diakinesis; × 1,625. *H*, Macrospore mother cell, heterotypic equatorial plate stage; × 850. (Drawings made with camera lucida at table level.

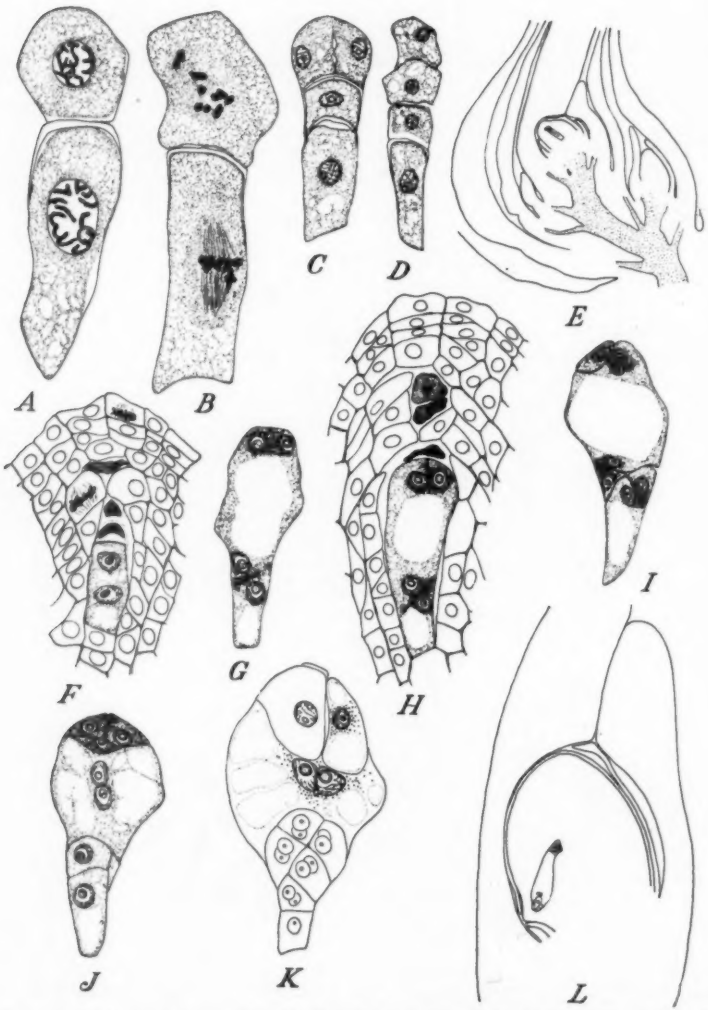

FIGURE 2.—*Euchlaena mexicana: A*, Interkinesis, nuclei preparing to divide; × 850. *B*, Homoeotypic division: × 850. *C* and *D*, Tetrads showing arrangement of spores; × 425. *E*, Longitudinal section through pistillate flower, ovule contains a tetrad of spores; × 45. *F*, Portion of ovule with two-nucleate embryo sac and 3 disintegrating spores; × 425. *G*, Four-nucleate embryo sac, cell-plate formation; × 425. *H*, Portion of ovule with four-nucleate embryo sac, later stage of cell-plate formation; × 425. *I*, Three-celled, eight-nucleate embryo sac showing cell-plate formation; × 425. *J*, Seven-celled, eight-nucleate embryo sac; × 425. *K*, Embryo sac showing increase of antipodal cells and the beginning of starch formation; × 425. *L*, Ovule with maturing embryo sac; shows development of integuments; × 45. (Drawings made with camera lucida at table level.)

Macrosporogenesis

The macrospore mother cell is about twice as long as wide at the onset of meiosis (fig. 1, *B*, *C*). It is somewhat flattened at both ends and wider in the apical portion than at the base. The nucleus, which may be either near the apical end of the cell or in the midregion, passes through the phases characteristic of the heterotypic division (fig. 1, *C* to *H*). Ten pairs of chromosomes are present at diakinesis (fig. 1, *F*), one of which is closely associated with the nucleolus (fig. 1, *G*). The chromosomes at this stage vary in length so that the longest pair is approximately twice as long as the shortest. The spore mother cell increases in size during the heterotypic prophases so that it is fully three times as long as wide at diakinesis.

The cytoplasm of the spore mother cell remains finely vacuolate while the nucleus is passing through the prophases. Toward the end of diakinesis, however, the cytoplasm at the ends of the cell becomes more vacuolate and a denser zone appears immediately surrounding the nucleus (fig. 1, *F*). The heterotypic spindle, whose axis is parallel to the longitudinal axis of the cell, is surrounded by a dense layer of cytoplasm (fig. 1, *H*). At the conclusion of the heterotypic division the spore mother cell is unequally divided by a cell plate, the apical (micropylar) daughter cell being about half as long as the basal (chalazal) cell. A thick wall is laid down by the chalazal cell at its micropylar end during the interim between the two meiotic divisions. The micropylar cell remains thin-walled (fig. 2, *A*).

The axis of the homoeotypic spindle in the chalazal daughter is cell longitudinal; the dense layer of cytoplasm immediately surrounding the spindle is not as apparent as in the preceding division (fig. 2, *B*). This cell likewise divides unequally, and a thick wall is formed on the micropylar end of the basal cell (fig. 2, *C*, *D*). The axis of the spindle in the micropylar cell formed as a result of the first meiotic division may be either longitudinal, transverse, or at an oblique angle to the long axis of the ovule. The nuclear division in this cell lags somewhat behind that in the chalazal cell (fig. 2, *B*). Two thin-walled daughter cells of approximately equal size are formed (fig. 2, *C*, *D*). The four daughter cells resulting from the meiotic divisions thus consist of one elongate chalazal cell which becomes the functional spore and three cells nearly equal in size which ultimately disintegrate. Not more than one spore tetrad was observed in any ovule (fig. 2, *E*).

Development of the Embryo Sac

The cytoplasm of the functional macrospore becomes highly vacuolate toward the chalazal end (fig. 2, *C*, *D*), and ultimately a large vacuole is formed in this region. Shortly after the first nuclear division in the formation of the gametophyte a second vacuole is formed between the two nuclei (fig. 2, *F*). This vacuole becomes much larger than the first, and as a result the nuclei are widely separated. Both daughter nuclei now divide and a distinct cell plate is formed across each spindle (fig. 2, *G*). The embryo sac is thus divided into three regions, namely, a large central region containing two nuclei (one daughter nucleus from each pair) and a large central vacuole (fig. 2, *H*); a chalazal region containing one nucleus and a basal vacuole; and an apical region with one nucleus and finely vacuolate cytoplasm.

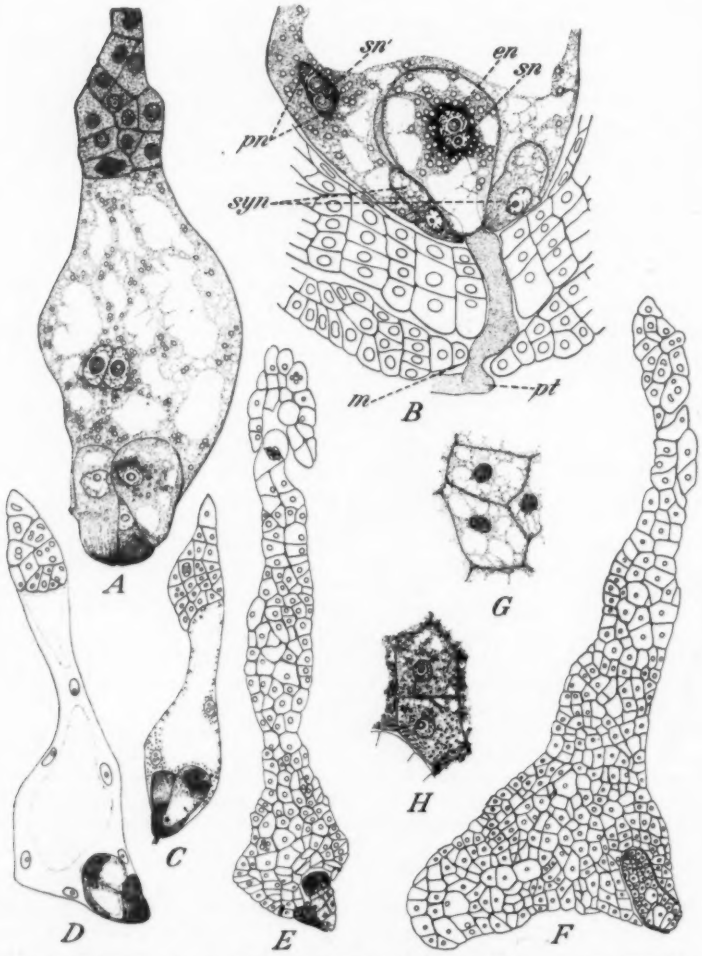

FIGURE 3.—*Euchlaena mexicana: A*, Embryo sac showing starch in egg and endosperm mother cell; × 425. *B*, Apical portion of embryo sac at time of fertilization; tip of pollen tube (*pt*) lies beneath fertilized egg; *pn*, polar nuclei fusing; *sn'*, male gamete nucleus fusing with polar nuclei; *en*, egg nucleus; *sn*, male gamete nucleus fusing with egg nucleus; *syn*, synergids; *m*, micropyle; × 425. *C*, Embryo sac shortly after fertilization showing zygote and two-nucleate endosperm; × 175. *D*, Embryo sac with a two-celled embryo and accompanying suspensor cell. A multinucleate endosperm is present; × 175. *E*, Longitudinal section through endosperm and young embryo with multicellular suspensor (*s*). Note persistent antipodals and synergids; × 175. *F*, Same as *E*. Later stage showing development of endosperm; × 175. *G*, A few endosperm cells from the antipodal region. Highly vacuolate cytoplasm with little evidence of storage materials; × 425. *H*, A few cells of the endosperm lying adjacent to the embryo showing dense cytoplasm and abundant storage materials; × 425. (Drawings made with camera lucida at table level.)

The cell plates formed across the spindles of the second division become increasingly distinct as additional cell plates are formed across the spindles of the third division (fig. 2, *I*). The apical region delimited by the second division divides to form the two synergids. The basal region divides to form two antipodals, one in which the cytoplasm is finely vacuolate and one having a large basal vacuole. The nucleus in the apical portion of the central region divides and a cell plate is formed, cutting off an egg with dense cytoplasm and leaving one nucleus in the central region. Thus the egg nucleus is the sister of one of the polar nuclei. The basal nucleus of the central region likewise divides, and a cell plate cuts off a third antipodal cell, leaving one daughter nucleus in the central region. The large middle cell now contains two nuclei separated by a central vacuole. This vacuole soon disintegrates into a number of smaller ones and the two polar nuclei move to the midregion of the cell, ultimately coming to lie closely adjacent in the vicinity of the egg apparatus (fig. 2, *J*). These nuclei are embedded in a central strand of cytoplasm that extends from the antipodals to the apex of the cell. The seven-celled, eight-nucleate embryo sac now present is approximately three times as long as, and correspondingly wider than, the functional macrospore before the division of its nucleus.

The embryo sac grows to twice its original length (fig. 3, *A*). During its period of growth the antipodal cells divide, reaching a number of 30 or more. These cells have distinct walls and are usually uninucleate, but occasionally a cell with two to four nuclei is present. The nuclei stain heavily and the cytoplasm is finely vacuolate.

The egg apparatus consists of three cells, the egg and two synergids. All of these are somewhat pear-shaped, the smaller ends extending toward the micropyle. The cells increase greatly in size during the final growth period of the embryo sac. The nucleus of each synergid lies in the midregion of the cell just above a large basal vacuole. The apical cytoplasm stains heavily and a conspicuous filiform apparatus is present at the micropylar end of each synergid (fig. 3, *A*).

The egg nucleus is embedded in dense cytoplasm in the midportion of the cell. The cytoplasm except in the vicinity of the nucleus is vacuolate, and a large vacuole occupies the micropylar end of the egg. The primary endosperm cell of the embryo sac remains binucleate. In its cytoplasm, as the cell matures, many small and several large vacuoles are formed. The two polar nuclei, lying in the apical portion of the cell, are in close contact. Many starch grains are present both in this cell and in the egg; there appear to be none in the synergids and antipodals. No starch is to be seen in the newly formed seven-celled embryo sac (fig. 2, *J*). As the sac develops, however, starch grains are formed in the dense cytoplasm immediately surrounding the polar nuclei and in that about the egg nucleus (fig. 2, *K*). The egg enlarges greatly just prior to fertilization, so that it extends into the embryo sac for some distance beyond the basal ends of the synergids.

Fertilization and Development of the Embryo

Fertilization takes place between 15 and 20 hours after pollination. The pollen tube enters the embryo sac between the synergids; usually neither of these is disorganized at this time (fig. 3, *B*, *C*). One of the

male nuclei (*sn*) fuses with the egg nucleus (*en*), and the other (*sn'*) fuses with the two polar nuclei (*pn*). Both fusions are shown in figure 3, *B*. The end of the pollen tube (*pt*) can be seen in the micropyle and extending between the apical cells of the nucellus. Its rounded tip lies beneath the zygote. The tube is constricted in the region where it is surrounded by the apices of the synergids and zygote. The dense cytoplasm of the primary endosperm cell becomes reorganized at or shortly after fertilization so that it forms a thin layer lining the periphery, a large vacuole occupying the central region of the cell. It is difficult to analyze the details of the zygote nucleus because of the many starch grains present in the immediately adjacent dense cytoplasm. Much starch is present also in the endosperm cytoplasm, especially in the region of the fusing nuclei. Division of the endosperm nucleus occurs almost immediately after fertilization; two and often four nuclei are formed before the complete disintegration of the remnants of the pollen tube.

Four to eight nuclei are present in the endosperm before the zygote divides; by the time a four-celled embryo is formed 25 to 30 endosperm nuclei lie in the thin peripheral layer of dense cytoplasm (fig. 3, *D*). Soon cell walls are formed between the endosperm nuclei, and large highly vacuolate cells are formed. Those endosperm cells in the neighborhood of the embryo now divide rapidly so that many small cells are present in this region, whereas the endosperm cells in the region of the antipodals are large and highly vacuolate. The endosperm contains many cells by the time the embryo reaches the stage shown in figure 3, *E*, in which the suspensor (*s*) is well differentiated. The endosperm develops at the expense of the nucellus, which in time is completely destroyed with the exception of the epidermis, which becomes a part of the seed coat. From this time on growth of the embryo is particularly active in two directions, toward the antipodals, which form a basal cap, and toward the nucellus at a right angle to the longitudinal axis of the embryo.

In material collected 5 days after pollination, the outgrowth of the endosperm directly opposite the embryo is more pronounced and the whole mass of endosperm has greatly increased in size. The peripheral layer of endosperm cells at this stage is conspicuous because of a differential staining reaction. Very little storage material is observed in the endosperm (fig. 3, *A*), except in those cells immediately adjacent to the embryo. The cytoplasm of these cells is dense and stains heavily and in some cells starch grains are present (fig. 3, *H*). The synergids are usually both intact although somewhat shrunken. They lie immediately beneath the suspensor shown in figure 3, *F*. The antipodal cells are well formed at this stage and show no signs of disintegration.

ZEA MAYS

The ovule of corn develops in a manner similar to that described for teosinte. The form at maturity is likewise similar. A hypodermal cell in the apical region becomes the primary archesporial cell (fig. 4, *A*). This cell does not divide to form a primary parietal and primary sporogenous cell but functions as a macrospore mother cell. The longitudinal axis of the heterotypic spindle is parallel with the long axis of the cell (fig. 4, *B*). After the first meiotic division two unequal daughter cells are formed, the chalazal cell being about twice

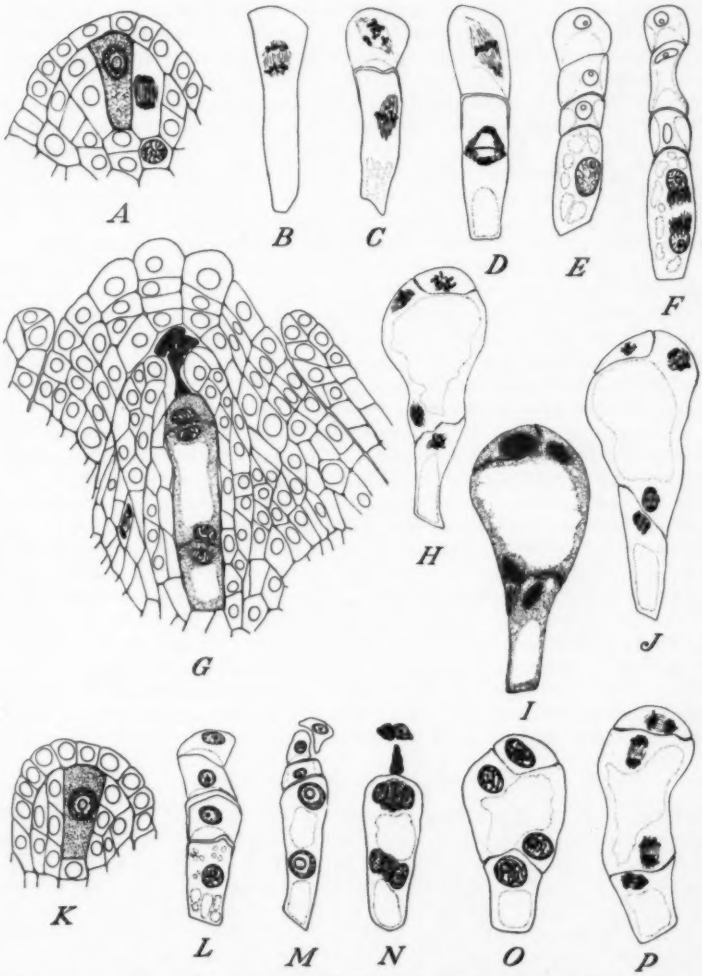

FIGURE 4.—*Zea mays: A*, Apical portion of ovule showing archesporial cell and dividing nucellar cells. *B*, Macrospore mother cell, heterotypic anaphase. *C*, Homoeotypic division, equatorial plate in basal cell and an earlier stage of division in apical cell. *D*, Same as *C*. Cell-plate formation in basal cell and equatorial plate in apical cell. *E*, Tetrad of spores. Large basal cell is the functional spore. *F*, Two-nucleate embryo sac with three disintegrating spores. *G*, Apical portion of ovule showing a four-nucleate, three-celled embryo sac and three disintegrating spores. *H*, Third division in embryo sac. Chromosomes advancing to the equatorial plates. *I*, Sames as *H*. Equatorial plate stage. *J*, Same as *H*. Telophase showing cell-plate formation. All × 425.

Zea mays × *Euchlaena mexicana: K*, Apical portion of ovule with archesporial cell. *L*, Tetrad of spores. *M*, Two-nucleate embryo sac and three disintegrating spores. *N*, Four-nucleate embryo sac showing cell-plate formation. *O*, Four-nucleate, three-celled embryo sac. *P*, Third division in embryo sac, telophase. All × 425. (Drawings made with camera lucida at table level.)

as long as the micropylar one. A thick wall is formed at the apex of the chalazal cell immediately after this division.

During the homoeotypic division the longitudinal axis of the spindle in the chalazal cell is approximately parallel to the long axis of the cell, whereas the axis of the spindle in the micropylar cell is usually at an angle (fig. 4, *C*, *D*). Nuclear and cell division in the chalazal cell usually precede division in the micropylar cell. The chalazal cell divides unequally and the innermost is the larger of the two daughter cells (fig. 4, *E*). A thick wall is formed at the apex of the innermost cell. The micropylar cell divides so that the two daughter cells are approximately equal in size. Of the four spores now present, the innermost and largest becomes the functional spore (fig. 4, *E*). The other three, approximately equal in size, ultimately disintegrate.

The nucleus of the functional macrospore divides (fig. 4, *F*). The daughter nuclei pass to opposite ends of the sac and then divide. Cell plates are laid down across the spindles of this second division in such a way that a three-celled embryo sac is formed consisting of a uninucleate cell at each end and a large central binucleate cell (fig. 4, *G*). By the time the embryo sac has reached this stage the three nonfunctional spores are in an advanced stage of disintegration.

The embryo sac is fully twice as long at the time of the third division as was the functional macrospore. The sac has likewise increased in diameter in the micropylar region, the diameter here being three times that of the spores. In consequence, the embryo sac is somewhat pear-shaped at the time of the third division (fig. 4, *H*, *I*, *J*). Cell plates are laid down across the spindles of the third division (fig. 4, *J*), in a manner similar to that described for teosinte, a seven-celled, eight-nucleate embryo sac being formed. The egg nucleus is a sister of one of the polar nuclei. Later the antipodal cells increase in number (30 to 40 or more) by division and a large amount of starch is stored in the egg and the primary endosperm cell. Many of the antipodal cells contain two or more nuclei each. This antipodal tissue remains as a well-developed structure in almost mature grains of corn which were collected 25 days after pollination.

ZEA MAYS × EUCHLAENA MEXICANA

The process of macrosporogenesis in *Zea mays* × *Euchlaena mexicana* is essentially similar to that found in both parents. The primary archesporial cell becomes the spore mother cell. (fig. 4, *K*). Four spores are formed as the result of the two meiotic divisions, the two toward the chalaza having thick walls at their micropylar ends (fig. 4, *L*). The innermost and largest spore becomes the macrospore and the others disintegrate. The developing macrogametophyte passes through stages similar to those described for teosinte (fig. 4, *M* to *P*). Conspicuous cell plates form across the spindles of the second division (fig. 4, *N*). The sac is now three-celled and four-nucleate (fig. 4, *O*). All four nuclei divide (fig. 4, *P*), cell-plate formation ensues, and an eight-nucleate, seven-celled embryo sac is the result. The antipodal cells divide so that 30 to 40 are present in the mature gametophyte. Starch grains are formed in the egg and in the primary endosperm cell. The starch is particularly abundant in the neighborhood of the egg nucleus and of the fusion nuclei.

DISCUSSION

The ovules of teosinte, corn, and the hybrid between them are amphianatropous at the time of maturity. True (*12*) described the ovule of corn as being campylotropous; Miller (*8*) and Randolph (*10*) found it to be of a modified campylotropous type; Weatherwax (*13*) reported it as anatropous. However, his figure of the ovule of *Coix* shortly after fertilization is similar to the ovules of *Euchlaena* and *Zea* as herein described.

The primary archesporial cell in the three forms studied functions directly as the sporogenous cell without further division. Weatherwax (*15*) described a periclinal division of the archesporial cell of corn forming a parietal cell and the macrospore mother cell. No cell wall is formed, however, according to him, and the parietal cell is "immediately consumed." The writer has examined a large number of preparations containing many stages in the development of the archesporial cell in order to find evidences of such a division. During the early stages of development of the ovule the nucellar cells adjacent to the archesporial cell divide periclinally in the manner shown in figure 4, *A*, but no evidence of a division of the archesporial cell was observed prior to the first meiotic division.

The spore mother cell divides to form four spores. The thick walls at the micropylar ends of the two innermost of the four spores are particularly conspicuous in teosinte and in the corn-teosinte hybrid. These walls take a deep purple color when stained with Delafield's haematoxylin and a bright pink color when the iodine-gentian violet combination is used. In no case observed were only three spores formed because of a failure of the completion of the homoeotypic division in the micropylar cell as described by Weatherwax (*15*).

The chalazal spore functions as a macrospore and the three apical spores disintegrate. Miller (*8*) reported that all four spores enter into the formation of the embryo sac. On the other hand, Weatherwax (*15*) found, as the writer has, that the chalazal spore alone continues development and the other three become disorganized. Brink (*4*), in a plant heterozygous for the waxy type of starch, found approximately a 1–1 segregation of the two types of starch in the embryo sac. This evidence excludes the possibility of all four macrospores entering into the formation of a macrogametophyte.

The development of the embryo sac is interesting because of the cell-plate formation across the spindles of the second division. In the material stained with Delafield's haematoxylin the plates are clearly differentiated. This fact, in addition to the position of the spindles of the third division, substantiates the observation of Weatherwax that one of the polar nuclei is a sister of the egg nucleus. Figure 2, *I*, showing cell-plate formation after the third nuclear division in teosinte, shows that the synergids on the one hand, and the egg nucleus and the upper polar nucleus on the other, represent sister nuclei. Schnarf (*11*) considers this as probably being the case in most, if not all angiosperms.

During the period of further maturation of the macrogametophyte the antipodal cells increase in number by division until 30 to 40 or more are present. These cells are of approximately the same size in teosinte and corn. Those of *Coix* (*17*) are fewer in number, much larger, and more highly vacuolate. This antipodal tissue remains

intact for a long period after fertilization, forming a cap at the apex of the endosperm. Material of teosinte 5 days after pollination shows well-developed antopodal cells as do also corn kernels collected 25 days after pollination. Weatherwax (*16*) and Randolph (*10*) found evidence of the presence of antipodal tissue just opposite the dent in fully matured corn kernels.

SUMMARY

Ovule development, macrosporogenesis, and the formation of the macrogametophyte are essentially similar in the annual variety of Florida teosinte, yellow dent corn, and a corn-teosinte hybrid.

Just before the appearance of the primordia of the integuments, a single hypodermal cell of the nucellus is differentiated as a primary archesporial cell. This cell functions as a macrospore mother cell.

The macrospore mother cell by two divisions produces a row of four macrospores.

The chalazal spore becomes the embryo-sac mother cell and the three micropylar spores disintegrate.

After the second nuclear division in the embryo sac, cell plates are formed across the spindles, producing a three-celled embryo sac.

A third nuclear division, followed by cell division leads to the formation of an eight-nucleate, seven-celled embryo sac. The two synergid nuclei are sister nuclei; so are the egg nucleus and one polar nucleus.

The antipodal cells continue to divide during the course of maturation and growth of the embryo sac so that 30 to 40 cells or more are formed. These persist as a definite tissue in the developing seed.

During the later stages of the maturation of the embryo sac starch is stored in the egg and the primary endosperm cell.

The ovule at the time of fertilization is amphianatropous in form.

Fertilization occurs in teosinte between 15 and 20 hours after pollination.

The synergids are not disorganized as a result of the entrance of the pollen tube. They persist in the region of the micropyle for 4 to 5 days and then disintegrate.

LITERATURE CITED

(1) Arnason, T. J.
1936. cytogenetics of hybrids between zea mays and euchlaena mexicana. Genetics 21: 40–60, illus.

(2) Beadle, G. W.
1932. the relation of crossing over to chromosome association in zea-euchlaena hybrids. Genetics 17: 481–501, illus.

(3) ———
1932. studies of euchlaena and its hybrids with zea. I. chromosome behavior in euchlaena mexicana and its hybrids with zea mays. Ztschr. Induktive Abstamu. Vererbungslehre 62: [291]–304, illus.

(4) Brink, R. A.
1925. mendelian ratios and the gametophyte generation in angiosperms. Genetics 10: 359–394.

(5) Emerson, R. A.
1924. control of flowering in teosinte. short-day treatment brings early flowers. Jour. Heredity 15: 41–48, illus.

(6) ——— and Beadle, G. W.
1932. studies of euchlaena and its hybrids with zea. ii. crossing over between the chromosomes of euchlaena and those of zea. Ztschr. Induktive Abstam. u. Vererbungslehre 62: [305]–315.
(7) Guignard, L.
1901. la double fécondation dans le mais. Jour. Bot. 15: [37]–50.
(8) Miller, E. C.
1919. development of the pistillate spikelet and fertilization in zea mays l. Jour. Agr. Research 18: 255–266, illus.
(9) Poindexter, C. C.
1903. the development of the spikelet and grain of corn. Ohio Nat. 4: 3–9, illus.
(10) Randolph, L. F.
1936. developmental morphology of the caryopsis of maize. Jour. Agr. Research 53: 881–916, illus.
(11) Schnarf, K.
1936. contemporary understanding of embryo sac-development among angiosperms. Bot. Rev. 2: 565–585, illus.
(12) True, R. H.
1893. on the development of the caryopsis. Bot. Gaz. 18: 212–226, illus.
(13) Weatherwax, P.
1916. morphology of the flowers of zea mays. Bull. Torrey Bot. Club 43: 127–144, illus.
(14) ———
1917. the development of the spikelets of zea mays. Bull. Torrey Bot. Club 44: 483–496, illus.
(15) ———
1919. gametogenesis and fecundation in zea mays as the basis of xenia and heredity in the endosperm. Bull. Torrey Bot. Club 46: 72–90, illus.
(16) ———
1926. persistence of antipodal tissue in the development of the seed of maize. Bull. Torrey Bot. Club 53: 381–384, illus.
(17) ———
1930. the endosperm of zea and coix. Amer. Jour. Bot. 17: 371–380, illus.

U. S. GOVERNMENT PRINTING OFFICE: 1937